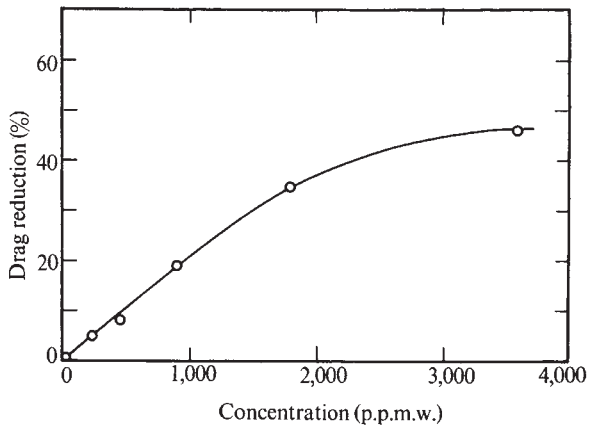

Fig. 1 Drag reduction of a solution of 'Scotch' tape in petroleum ether. Solvent Reynolds number was 60,000.

to be the fractional increase in the flow rate of the sample over that obtained for the solvent alone.

In view of Cossar's results it was initially anticipated that an extremely dilute solution, obtained from soaking 'Scotch' tape in water, would give rise to a large drag reduction. But early attempts to observe the Toms effect with such solutions proved unfruitful, and it was only after prolonged soaking of the tape that any effect was observed. For example, after soaking 0.02 m^2 of 'Scotch' tape in 200 ml. of water for 40 days, the resulting solution showed a drag reduction of 19% compared with water when ejected at a Reynolds number of 8,200. With another proprietary brand of adhesive tape ('Sellotape') the same procedure gave a drag reduction of 8% which rose to 20% after 80 days of immersion. In connexion with these measurements it is perhaps worth noting that: (i) the viscosity of the 'Scotch'-tape solution, measured under laminar flow conditions, was about 6% greater than that of water at the same temperature; and (ii) the drag reduction remained unchanged when the adhesive tape solutions were passed through filter paper (Whatman No. 1 grade) before being tested.

To obtain a solution of known concentration, the gum from a sample of 'Scotch' tape was completely dissolved in a "good" solvent (petroleum ether). The properties of this solution are not expected to differ greatly from those of the corresponding aqueous solution. The values of the drag reduction obtained at various dilutions are shown in Fig. 1. At a concentration of 3,600 p.p.m.w., when the viscosity of the solution was 50% greater than that of the solvent, the drag reduction was 46%. The results in Fig. 1 suggest that, compared with some drag reducing compounds, the concentrations of the present solutions need to be quite large in order to produce substantial drag reduction. On the other hand, if our suggestions regarding Cossar's experiments are correct, it would seem from his results that only extremely small quantities of additive can have large influences on a turbulent boundary layer when it is supplied from the wall. Accordingly it might be highly effective, for drag reduction, to coat surfaces with such compounds, which, because of their poor solubility in water, could have a fairly long period of effectiveness.

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BIOLOGICAL SCIENCES

Quantitative Evidence for Protein Denaturation as the Cause of Thermal Death

The specific cause of the death rates of unicellular organisms and poikilothermic animals at high temperatures is not known for certain[1]. We wish to report a good numerical correlation between certain thermodynamic parameters in protein denaturation and death rates, where these have been reported, for these organisms. A similar correlation may exist between the thermal denaturation of other biopolymers, such as DNA and RNA, and thermal death, but in the absence of adequate data we cannot consider this possibility further.

At sufficiently high temperatures (>315 K) most proteins denature with pseudo-first order kinetics. This implies that the renaturation rate is slow enough to be negligible, and the rate is described by $dn/dt = -k_D n$. The first order denaturation rate constant, k_D, varies exponentially with temperature and is usually described by the absolute rate theory equation[2]

$$k_D = \kappa \frac{k_B T}{h} \exp[\Delta S^{\ddagger}/R] \times \exp[-\Delta H^{\ddagger}/RT] \qquad (1)$$

where $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the activation entropy and activation enthalpy respectively.

We have found evidence from the work of Williams and Milby[3] on the thermal denaturation of rhodopsins that, for the proteins tested, the $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ values were related by the simple linear equation[4]

$$\Delta S^{\ddagger} = a\Delta H^{\ddagger} + b \qquad (2)$$

The existence of such a relationship constitutes a compensation law behaviour, because variations of $\Delta H^{\ddagger}$ in the negative exponential of equation (1) are partially compensated by parallel variations of $\Delta S^{\ddagger}$ in the positive exponential in their effects on k_D. (At the temperature $T = 1/a$, the compensation is exact and the denaturation rate is independent of the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. This temperature, T_c, is the compensation law temperature.) Such compensation law behaviour occurs in a number of physical and chemical processes[5-9] for which *ad hoc* explanations have been suggested. So far there is no compelling evidence that such a rule as equation (2) can be derived from general thermodynamic principles. Exner[10] has pointed out that, in certain limited conditions, a compensation law may occur accidentally, and he has provided a set of criteria for judging the reliability of the rule. In some of the cases described here, such criteria have been used and satisfied.

We tested the generality of such a compensation law using the compilation of thermodynamic data on protein denaturations from Joly[11]. Fig. 1 presents all these data, plotted as $\Delta S^{\ddagger}$ versus $\Delta H^{\ddagger}$, with no selection. All the data cluster about a linear regression line. From a least squares fit of the data points, the line yields a value of $T_c (= 1/a)$ of 329 K and a value of $b = -64.9$ calories/mol × K (an equally valid intercept could be the value of $\Delta H^{\ddagger}$ when $\Delta S^{\ddagger} = 0$; this yields $\Delta H^{\ddagger} = 21.3$ kcalories/mol). The corresponding values for the rhodopsins are: $T_c = 334$ K and $b = -66$ calories/mol × K. Several of the proteins are represented by a number of points, each referring to a specific *p*H or ionic strength of the solution. Therefore, although the $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ values for a given protein may vary depending on the experimental conditions, the values always fall on the same line.

Sukhorukov and Likhtenshtein[12] have published a plot similar to Fig. 1 for a large number of proteins, which overlap only partially the proteins of our figure. The values we have estimated from their plot are: $T_c \simeq 325$ K, $b \simeq -70$ calories/mol × K, which are closely similar to ours. They have also included proteins and viruses in the dry state, as well as proteins in alcohol–water mixtures, and in D_2O solution, all of which still fall on the compensation law line.

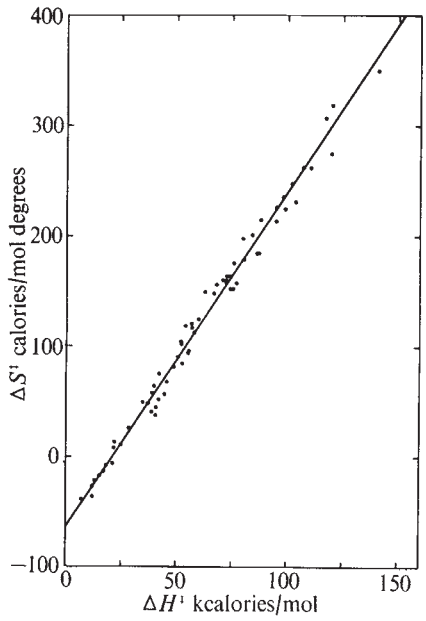

Fig. 1 A plot of the activation entropy, $\Delta S^{\ddagger}$, versus the activation enthalpy, $\Delta H^{\ddagger}$, for protein denaturation (after Joly[11]). The regression line is a least squares fit to the data, and the close clustering to the line indicates the validity of the compensation law (equation 2). The constants of the line are: $T_c = 329$ K, and $b = -64.9$ calories/mol × K.

We conclude that virtually all proteins[7], in a variety of conditions, denature at elevated temperatures according to the compensation law of equation (2), with the same value of T_c and b.

Lumry (personal communication) has called our attention to the work of van Uden and his co-workers[13] who have reported compensation law behaviour in the thermal killing of yeasts. Here again (1) the death rates followed pseudo-first order kinetics with respect to time; (2) the specific thermal death rate, k_D, depended exponentially on temperature; and (3) the $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ values obtained were correlated according to equation (2). From their data we have calculated the values of the compensation law constants as $T_c = 325$ K and $b = -64.5$ calories/mol × K.

We have attempted to pursue this correlation with other unicellular organisms such as viruses and bacteria. Usable data in the literature are surprisingly scant, but Barnes *et al.*[14] have carefully investigated this compensation law in the thermal inactivation of the Sindbis virus. Their data again meet the three criteria described above, and they calculate $T_c = 330$ K; while we calculate $b = -64$ calories/mol × K. In the case of bacteria, data are available on *Pseudomonas fragi*[15], *Staphylococcus aureus*[16,17], and two strains of *Salmonella*[17] which also meet our three criteria. The values we have calculated for the compensation law constants are $T_c = 331$ K and $b = -65$ calories/mol × K.

We have plotted all the points for the yeasts, virus and bacteria in Fig. 2. In Table 1 we collect all the values of the constants for comparison. The unicellular organisms have a specific death rate for thermal killing which follows a compensation law with constants which are in very good agreement with the constants for thermal denaturation of proteins. We suggest that this is good quantitative support for the general hypothesis that protein denaturation is the cause of thermal death in unicellular organisms. Such a statement, however, does not allow the identification of any specific protein as the limiting cause, because the cells will have different $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ values in different environmental conditions, and any specific protein will exhibit a class of $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ values in different environmental conditions *in vitro*. It may be possible, though difficult, to find an isolatable protein, or group of proteins, the $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ values of which *in vitro* coincide with those of the cell for the same conditions, and draw the inference that this (these) is (are) the limiting protein(s). The viruses, constituted with a small number of proteins, recommend themselves for such a study.

Table 1 Comparison of Compensation Law Constants, T_c and b, from : $\Delta S^{\ddagger} = a\,\Delta H^{\ddagger} + b$; $T_c = 1/a$

	T_c (K)	b (calories/mol × K)
Proteins	329	−64.9
Virus	330	−64
Yeasts	325	−64.5
Bacteria	331	−65

In a cell, a protein i is manufactured with a creation rate c_i, and has a denaturation rate, $k_{D_i} n_i$ (again we ignore renaturation, although it could be subsumed under c_i); therefore we have:

$$\frac{dn_i}{dt} = c_i - k_{D_i} n_i \qquad (3)$$

If a steady state could occur, then the number of molecules of protein i is $n_i = c_i / k_{D_i}$. The creation rate c_i must be assumed to be a complex function of both the genetic structure and environmental conditions. For a population of cells to die with pseudo-first order kinetics, the temperature must be high enough that the denaturation rate overwhelms the ability of the cell to create the proteins that are limiting, and $dn_i/dt = k_{D_i} n_i$. If N is the number of viable cells, then our hypothesis requires that $dN/dt = -k_{D_i} N$. It is not obvious why the k_{D_i} for the cell population should be identical with k_{D_i} for the protein population. For example, the assumption that a threshold number of proteins, i, is essential for cell viability would lead to a single time instant of death for all the cells of the population, rather than a constant probability of death as the kinetics require. It can be shown mathematically that at least three hypotheses connecting protein denaturation with cell death could lead to the appropriate kinetics and thermodynamics. These are (*a*) that there is a statistical distribution of protein concentration thresholds in the population of cells; (*b*) that the stress on the cell to create protein at a rate sufficient to compensate the denaturation rate is the cause of death; and (*c*) that the stress on the cell to prevent the accumulation of denatured protein

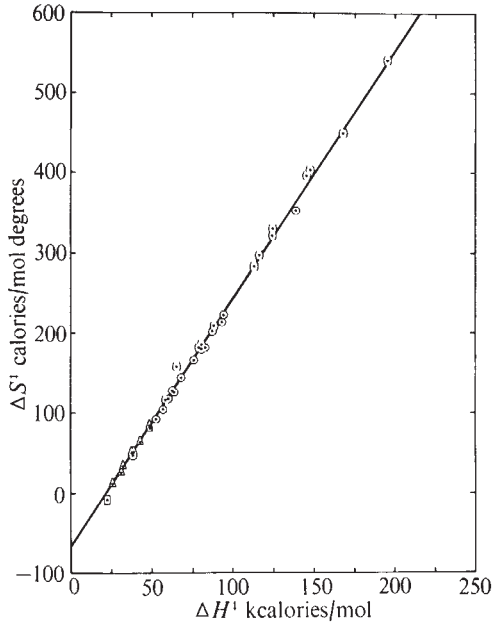

Fig. 2 A plot of the activation entropy, $\Delta S^{\ddagger}$, versus the activation enthalpy, $\Delta H^{\ddagger}$, for thermal killing of various organisms: ·, yeasts (after van Uden *et al.*[13]; ○, bacteria (after Luedecke[15], Walker[16], and Beamer and Tanner[17]); △, virus (after Barnes *et al.*[14]); and □, *Drosophila m.* (after Strehler[18]). The constants of the line are: $T_c = 325$ K and $b = -66$ calories/mol × K.

debris kills the cell. There are undoubtedly other possible hypotheses which should be tested.

In Fig. 2 we have plotted a single point on the line which refers to the multicellular poikilotherm, *Drosophila melanogaster*, after the data of Strehler[18]. We have replotted his death rate curves to obtain the first order kinetic plots versus time. These k_Ds do show an exponential dependence on the temperature; and using equation (1), we have calculated the $\Delta S^{\ddagger}$ for these data. The datum point falls on the line close to the $\Delta S^{\ddagger} \cong 0$ value. Strehler[1] has pointed out that a number of other studies on arthropods (*Drosophila*, *Pinus tectus* and *Daphnia*) have shown almost identical activation energies for the rate of ageing (as measured by the mortality rate) and these would yield data points close to $\Delta S^{\ddagger} \approx 0$, according to our estimates. We can now speculate that, if this is so, it has at least two explanations. First, the evolutionary jump from unicellular to multicellular organisms can be represented on the compensation law plot as the convergence of the $\Delta H^{\ddagger}$ values to the lowest value consonant with $\Delta S^{\ddagger} \geq 0$. That means that the arthropods have accepted a higher thermal death rate still consistent with survival of the species, for a smaller variation of the thermal denaturation rate of the limiting proteins with ambient temperature changes. Second, Sukhorukov and Likhtenshtein[12] have shown that many dry proteins fall on the compensation law plot at $\Delta S^{\ddagger} \approx 0$, and that hydration of these proteins causes the $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ values to move up the line. $\Delta S^{\ddagger} \approx 0$ may be the smallest value that can be reasonably expected in biological circumstances; therefore one could argue that in going up the evolutionary scale, "dry" proteins are first encountered in vital roles at the level of multicellular organisms. Because these proteins denature fastest, they represent the rate limiting step in the death of these organisms. These latter arguments are purely speculative.

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Note added in proof. A fourth hypothesis connecting protein denaturation and cell death was proposed by Rahn in 1929 (Rahn, O., *Bact Rev.*, **8**, 1; 1944). According to him, "a logarithmic order is possible only when death is due to the destruction of a single protein molecule in the cell". We find this the most attractive alternative, with some interesting ramifications which we will develop later.

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Preparation and Characterization of an Arylsulphatase insolubilized on Porous Glass

THE enzyme sterol sulphate sulphohydrolase (EC 3162) is used for the hydrolysis of steroid conjugates before analysis of body fluids for total oestrogens; but it is rather costly. The availability of a re-usable derivative of this enzyme which would be stable for a long time might be of value to the clinical chemist. I therefore decided to prepare an insolubilized derivative of this enzyme and determine its properties.

The enzyme was purchased as a mixture of β-glucuronidase/arylsulphatase (Boehringer). The arylsulphatase activity of the commercial preparation was 2.6 IU/ml. of enzyme solution. The insoluble enzyme was prepared as previously described[1-3]. Porous glass used for this study was 96% silica particles, pore diameter 550 Å, 40/60 mesh particle size. The enzyme was coupled through azo linkage to the glass. The insoluble enzyme was assayed with 0.001 M *p*-nitrophenylsulphate dissolved in 0.25 M Na_2CO_3 buffer (*p*H 9.0). The assay was carried out using 25 ml. of substrate to which was added a known weight of enzyme derivative. The reaction was continued for 10 min after which a sample was taken and read spectrophotometrically at 410 nm. The reaction was carried out at 25° C. Activity of the final product was 0.31 IU/g of glass. All other studies were carried out by the column method.

One gram of derivative was packed into a small, glass, 1.0 cm wide, jacketed column to a height of 5.0 cm. Temperature was maintained at 6° C throughout these studies.

A *p*H profile was obtained by passing 1×10^{-5} M *p*-nitrophenylsulphate through the column at a flow rate of 0.67 ml./min. The column effluent was continuously monitored at 410 nm in a flowthrough colorimeter. The buffers used were 0.25 M acetate between *p*H 5.0 and 6.7 and carbonate between *p*H 7.8 and 10.5. The *p*H optimum lies between *p*H 9.0 and 9.5 (Fig. 1).

By passing substrate at increasing concentrations through the column while maintaining constant flow rate (0.67 ml./min), the values for K_m and V_{max} were obtained (Fig. 2). The apparent V_{max} for the insoluble enzyme was 1.67×10^{-8} mol/s. The apparent K_m was 1.57×10^{-3} M. These values were

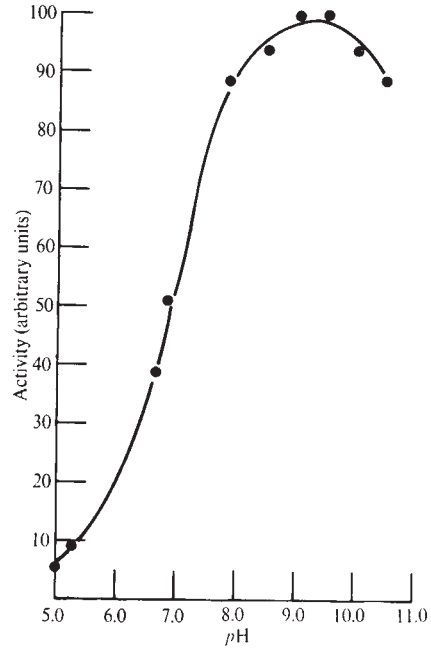

Fig. 1 *p*H profile using 1×10^{-5} M *p*-nitrophenylsulphate as substrate.