ACTA CHEMICA SCANDINAVICA 9 (1955) 1700—1710

# The Influence of Temperature on the Rate of Enzymic Processes

ESKIL HULTIN

*Institutet för organisk kemi och biokemi, University, Stockholm, Sweden*

Enzymes may be reversibly denatured at low temperatures. The enthalpy of activation $\Delta H^*$ (or Arrhenius' energy of activation $\mu$), the enthalpy of denaturation $\Delta H$, and the temperature for half denaturation $t_{\frac{1}{2}}$ can easily be computed from measurements of the activity at various temperatures. Earlier computations of the heat of activation for enzymic processes, founded on measurements done in a comparatively short interval of temperature, gave probably not the true value of the heat of activation but the sum of the true value and part of the negative value of the heat of denaturation. For the hydrolysis of tributyrin with pancreatic lipase at pH 8.1 $\Delta H^* = 4\,700$ cal/mole, $\Delta H = -27\,000$ cal/mole, and $t_{\frac{1}{2}} = 3$ °C. For the hydrolysis of saccharose with yeast saccharase at pH 4.45 $\Delta H^* = 10\,000$ cal/mole, $\Delta H = -51\,000$ cal/mole, and $t_{\frac{1}{2}} = -4$ °C. For the hydrolysis of casein with trypsin at pH 8.1 $\Delta H^* = 11\,000$ cal/mole, $\Delta H = -53\,000$ cal/mole, and $t_{\frac{1}{2}} = 0$ °C.

The influence of temperature on the rate of chemical processes was successively interpreted by van't Hoff [1] in 1884, by Arrhenius [2] in 1889 and by Eyring [3,4] in 1935.

It is usual to express this influence in one of the following two ways. It can be given as the energy of activation $\mu$ according to Arrhenius' equation which is generally written

$$k = PZe^{-\mu/RT} \tag{1}$$

or

$$\frac{\mathrm{d} \ln k}{\mathrm{d}\,(1/T)} = -\frac{\mu}{R} \tag{2}$$

where $k$ is the rate constant, $P$ is the steric factor [5], $Z$ is the number of collisions, $\mu$ the energy of activation, $T$ the absolute temperature and $R$ the gas constant.

The influence of temperature can also be expressed as the enthalpy of activation $\Delta H^*$ according to Eyring's equation:

$$k' = \frac{\varkappa kT}{h}\, e^{\Delta S^*/R}\, e^{-\Delta H^*/RT} \tag{3}$$

where $k'$ is the specific reaction rate, $\varkappa$ is the transmission constant, $k$ is Bolzmann's constant, $h$ is Planck's constant, $\Delta S^*$ is the entropy of activation, and $\Delta H^*$ is the enthalpy of activation. The enthalpy of activation $\Delta H^*$ is usually calculated as $\Delta H^* = \mu - RT$ ($T$ is here the average of the temperatures at which the experiments for the determination were carried out), but it has recently been shown by the present author [6] that it can also be calculated according to the following equation:

$$\frac{\mathrm{d} \ln (k/T)}{\mathrm{d} (1/T)} = -\frac{\Delta H^*}{R} \tag{4}$$

by plotting the logarithms of the rate constants divided by the absolute temperatures *versus* the inverse values of the absolute temperatures, and fitting a straight line to the points; the enthalpy of activation is obtained as the negative value of the inclination of the line multiplied by the gas constant $R$.

It is thus rather simple to characterize the change of the reaction rate with temperature for most chemical reactions. However, in the case of enzymic reactions, if the logarithms of the reaction rates are plotted *versus* the inverse values of the temperatures, and if the temperature interval is not small, it has been observed that the points usually do not lie about a straight line. The results from such investigations have recently been reviewed by Johnson, Eyring and Polissar [7].

In most investigations concerning the heat of activation of enzymic reactions, the temperature interval has been so small that a deviation from a straight line could not be demonstrated with any certainty. Hence a straight line was fitted in the corresponding Arrhenius plot. The results thus obtained are, of course, valid only as apparent heats of activation for the interval in question. For a list of such results the reader is referred to the reviews by Eyring and Stearn [8] and by Sizer [9].

Two cases of deviations from a straight line may be considered here. In one, best known from the investigations on luciferase, the reaction rate increases with the temperature at low temperatures, passes a maximum and then decreases reversibly as the temperature increases. In the other case, which is known from several enzyme systems, the reaction rate successively decreases with the temperature more than would be expected if the heat of activation at ordinarily high temperatures remained constant when the temperature was lowered. Several explanations have been given for this behaviour of enzymic reactions.

For the first case mentioned, exemplified by luciferase, Johnson *et al.*[7,10] and Eyring [11] gave the explanation that the enzyme is denatured as the temperature increases; however, this denaturation is not irreversible as in most other systems described but reversible with a definite equilibrium constant. A reversible denaturation at high temperatures is also known from investigations on other enzymes, *e. g.*, trypsin [12].

In the second case, as already mentioned, the rate successively decreases with decreased temperature more than would be expected if the heat of activation remained constants. For this case Sizer [13] suggested that two straight lines should be fitted in the Arrhenius plot and gave the explanation that the

sharp break in the temperature relationship indicates that the kinetics of the enzyme action is very different above and below this point. This suggestion is similar to the earlier suggestion of Blackman, Pütter and Crozier (see Ref. [7,14]) for the influence of temperature on the rate of biological processes: there is supposed to be a catenary series of chemical reactions with their own values for the heat of activation $\mu$. Kistiakowsky and Lumry [15] upon a kinetic analysis concluded that the existence of sharp breaks is rather unlikely. In one case, in which the existence of a sharp break had been claimed — urease in the presence of sulfite ions — they found that the apparent activation energy rises gradually and not suddenly as the temperature is lowered, and that the change in the apparent activation energy can be explained by the temperature dependence of reversible inhibition of urease by sulfite ions. It may be mentioned here that as early as 1930 Buchanan and Fulmer [16], in discussing the influence of temperature on the rate of carbon dioxide production in alcoholic fermentation, drew attention to the wantonness of dividing a smooth curve into several pieces of straight lines.

Another explanation for the behaviour of enzyme systems at low temperatures has been suggested by Kavanau [17]: there may be reversible denaturation of an enzyme at low temperatures, comparable to the reversible denaturation of some enzymes at high temperatures. However, Kavanau, in his further treatment of this problem, immediately leaves this theory and gives a description of how to fit a complicated curve (including the incomplete gamma function) to the experimental results. For this curve-fitting, which has no clear theoretical significance [7], Kavanau makes use of three parameters, whereas Arrhenius' equation has only two (usually denoted $PZ$ and $\mu$), and hence Kavanau gets a curve which is close to the experimental points over a wide interval.

## A THERMODYNAMIC INTERPRETATION

In this paper, the present author suggests a reversible inactivation of enzymes at low temperatures (first surmised by Kavanau [17]) as an important cause for the temperature dependence of many enzymic processes *.

### Theory

Let $E_a$ denote the active form of the enzyme, $E_i$ the inactive form, and $K$ the equilibrium constant, and let brackets denote concentrations. It is reasonable to consider here only the case that one molecule of this active form of the enzyme gives one molecule of the reversibly inactive form.

We thus get the following equations:

$$E_a \rightleftharpoons E_i \tag{5}$$

$$\frac{[E_i]}{[E_a]} = K \tag{6}$$

$$\frac{[E_a]}{[E_a] + [E_i]} = \frac{1}{1 + K} \tag{7}$$

* After this paper was prepared, a paper was published by Maier, Tappel, and Wolman [22], who use the same theory for peroxidase and phosphatase.

From thermodynamics the following relation between an equilibrium constant $K$, the absolute temperature $T$, the gas constant $R$ and the enthalpy $\Delta H$ is well-known:

$$\frac{\mathrm{d} \ln K}{\mathrm{d}\, 1/T} = -\frac{\Delta H}{R} \tag{8}$$

If half of the total amount of enzyme is in the active form at the temperature $T_{\frac{1}{2}}$, which implies that the equilibrium constant has the value 1, then we get on integration of eqn. (8).

$$\ln K = -\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_{\frac{1}{2}}}\right) \tag{9}$$

and

$$K = \exp\left[-\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_{\frac{1}{2}}}\right)\right] \tag{10}$$

Now having considered the reversible inactivation of the enzyme, we shall turn to the enzymatically catalyzed reaction. For the influence of temperature on the rate of this reaction we have Arrhenius' and Eyring's equations, already given here as eqns. (1) and (3) which in this form are valid if all of the enzyme is active. If only part of the enzyme is active but the specific reaction rate is calculated for the total amount of enzyme, we get the following expression by combination of eqns. (1) and (7)

$$k = \frac{PZ}{1 + K}\, \mathrm{e}^{-\mu/RT} \tag{11}$$

and

$$k' = \frac{\varkappa kT}{h} \cdot \frac{\mathrm{e}^{\Delta S^*/R} \cdot \mathrm{e}^{-\Delta H^*/RT}}{1 + K} \tag{12}$$

This expression is mathematically equivalent to the expression which is valid for the influence of temperature on the rate of an enzymic reaction if there is a reversible denaturation of the enzyme at *high* temperatures (see *e. g.*, Ref. [7], eqn. (8, 4)), whereas the expression here is valid for the denaturation at low temperatures.

From eqns. (11) and (12) we get the following equations for the asymptote of the curve in an Arrhenius' plot or the plot suggested by the present author [6] by multiplying with $1 + K$, and by deriving logarithmically

$$\frac{\mathrm{d} \ln k\,(1 + K)}{\mathrm{d}\, 1/T} = -\frac{\mu}{R} \tag{13}$$

$$\frac{\mathrm{d} \ln k\,(1 + K)/T}{\mathrm{d}\, 1/T} = -\frac{\Delta H^*}{R} \tag{14}$$

## The computation of $\Delta H^*$, $\Delta H$, and $T_{\frac{1}{2}}$

The computation of $\Delta H^*$, $\Delta H$, and $T_{\frac{1}{2}}$ can be done by successive approximations. The procedure will be exemplified with the data of Sizer and Josephson [13] for the hydrolysis of tributyrin with pancreatic lipase in the presence of 36 % glycerol.

*Table 1. Example of the computation of the equilibrium constant* K *for the low temperature denaturation of lipase from Sizer and Josephson's* [18] *measurements.*

1. The temperature for the experiments. 2. The time in hours for 1 % hydrolysis of tributyrin. 3. The abscissa values for Figs. 1—3. 4. The ordinata values of the experimental plots in Figs. 1 and 3. 5. The differences between the ordinata values for the asymptote and the plots as read in Fig. 1. 6. The ordinata values for Fig. 2.

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| °C | $1/k$ | $10^4/T$ | log $k/T$ | log $(1+K)$ | log $K$ |
| 50.0 | 0.0183 | 30.94 | 0.228 — 1 | | |
| 40.0 | 0.0198 | 31.93 | 0.208 — 1 | | |
| 30.0 | 0.0325 | 32.99 | 0.006 — 1 | | |
| 20.0 | 0.0850 | 34.11 | 0.603 — 2 | | |
| 10.0 | 0.0800 | 35.32 | 0.645 — 2 | | |
| 5.0 | 0.133 | 35.95 | 0.432 — 2 | | |
| — 0.2 | 0.137 | 36.58 | 0.427 — 2 | | |
| — 4.5 | 0.550 | 37.22 | 0.830 — 3 | 0.774 | 0.693 |
| — 4.5 | 0.400 | 37.22 | 0.969 — 3 | 0.635 | 0.521 |
| — 5.0 | 0.50 | 37.29 | 0.873 — 3 | 0.724 | 0.633 |
| — 9.0 | 1.62 | 37.86 | 0.369 — 3 | 1.171 | 1.140 |
| —10.0 | 8.50 | 38.00 | 0.650 — 4 | 1.874 | 1.868 |
| —12.0 | 4.0 | 38.29 | 0.981 — 4 | 1.514 | 1.500 |
| —15.0 | 3.5 | 38.74 | 0.044 — 3 | 1.405 | 1.388 |
| —18.0 | 7.0 | 39.19 | 0.748 — 4 | 1.655 | 1.645 |
| —18.0 | 4.0 | 39.19 | 0.991 — 4 | 1.412 | 1.395 |
| —18.0 | 7.5 | 39.19 | 0.718 — 4 | 1.685 | 1.676 |
| —24.5 | 87.5 | 40.22 | 0.662 — 5 | 2.636 | 2.635 |
| —24.5 | 55.0 | 40.22 | 0.864 — 5 | 2.435 | 2.433 |

The values of log $k/T$ are plotted *versus* $1/T$ (if one wishes to get $\mu$ instead of $\Delta H^*$, the values of log $k$ are plotted *versus* $1/T$). As the temperature increases, the reversible denaturation at low temperatures decreases, and thus, in the diagram, the plots asymptotically approach a straight line. This asymptote is drawn by the eye (Fig. 1) as is the first estimation of $\Delta H^*$. The differences between the corresponding ordinates for the asymptote and the points at low temperatures are read in the diagram; they give the values of log $(1 + K)$ (*cf.* eqn. (14)). The values of log $K$ are calculated (Tab. 1) and plotted versus $1/T$ in another diagram (Fig. 2) (*cf.* eqn. (8)). A straight line is fitted to the points *, and the heat of denaturation $\Delta H$ is calculated from the inclina-

* It can be seen from Fig. 2 that one of the points lies remarkably distant from the line. If all points are considered for calculating the regression line, the standard deviation calculated [18] according to the formula $s_{y \cdot x}\sqrt{1 + 1/n + x^2/\Sigma x^2}$ at the abscissa of the points is 0.32, whereas the difference in ordinata for the point and the line is 0.714, which is $t = 2.22$ times the standard deviation. The probability for this distance in random sampling is 5 %. If the regression line is calculated without this point, the standard deviation at the abscissa of the point is 0.21 and the difference in ordinata for the point and the line is 0.80, which is $t = 3.7$ times the standard deviation. The probability for this distance in random sampling is between 1 and 0.1 %. We have thus statistical reasons for excluding this point from the calculations.

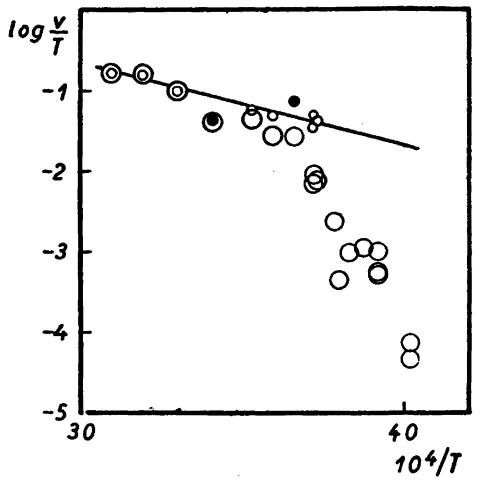


*Fig. 1. Fitting an asymptote to a plot[6] of the activity of lipase at various temperatures as measured by Sizer and Josephson [13] for the computation of the enthalpy of activation ΔH* and the equilibrium constant* K *between denatured and native lipase (*cf. *Table 1 and 2). Large rings: experimental values, log* k/T. *Small rings: experimental values corrected for reversible low temperature denaturation. Dots: experimental values which because of specified reasons were not regarded in fitting the line. In the first approximation, the straight line is fitted by the eye as the asymptote to the plots. In the second approximation, the straight line is fitted in the normal way to the corrected plots. Both log* k/T *and log* k(1 + K)/T *are thus plotted as ordinata values of log* V/T.

tion of the line in the usual way as the product of —1, the inclination, the gas constant $R = 1.9872$ cal · degree$^{-1}$ · mole$^{-1}$, and ln 10 = 2.3026. In this diagram the half activity temperature $T_{\frac{1}{2}}$ is also read as the temperature, for which the logarithm of the equilibrium constant has the value 0.

The second approximation is thereafter done as follows. The values of $\Delta H$ and $T_{\frac{1}{2}}$ are used for the calculation of the values of log $(1 + K)$ for the relatively high experimental temperatures. The values of log $K$ can be read in the diagram by extrapolation, and then log $(1 + K)$ and, finally, log $k(1 + K)/T$ are computed from these readings (Table 2). These values are now plotted versus $1/T$ (*cf.* eqn. (14)), and a straight line is fitted to the points *. (If one wishes to get $\mu$ instead of $\Delta H^*$, the values of log $k(1 + K)$ are plotted in an Arrhenius diagram.) This line is a better estimation of the true asymptote than the line previously drawn by the eye. The inclination is read (or calculated [18] by the method of least squares) and the value of the enthalpy of activation $\Delta H^*$ is calculated as usual (eqn. (14)). If the value for the inclination of the asymptote obtained after this approximation differs appreciably from the first estimation, the whole calculation is repeated with the new value.

* In this fitting two points have been excluded because of the following reasons. The plot of the experiment at 10 °C (fourth plot fom the left) lies remarkably lower than in Sizer and Josephson's Fig. 1, and there is probably a misprint in their Table 1. The other point which was excluded, at —0.2 °C (the seventh point from the left) is remarkably distant from the line; a statistical calculation gives the value $t = 10$, which corresponds to a probability less than 0.1 %.

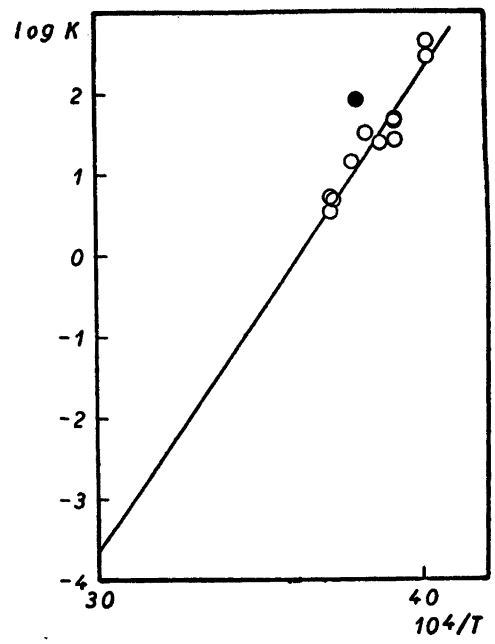


*Fig. 2. Fitting a straight line to the plots of the logarithm of the equilibrium constant K as obtained from Fig. 1 and Table 1 for the computation of the enthalpy of denaturation ΔH and for reading from the line the extrapolated values of log K for computing the corrected experimental values for plotting log k(1+K)/T; see Fig. 1. The dot gives one experimental value which for statistical reasons was not regarded in fitting the straight line.*

In this way the three parameters $\Delta H^*$, $\Delta H$ and $T_{\frac{1}{2}}$ are obtained. It is shown in Fig. 3 that a line with the parameters $\Delta H^* = 4\,700$ cal/mole,

*Table 2. Example of the computation of the corrections for the low temperature denaturation.*

1. The abscissa values for Figs. 1 and 2. 2. The ordinata values read in Fig. 2. 3. The correction term. 4. The ordinata values for the corrected experimental plots in Fig. 1.

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| $10^4/T$ | log $K$ | log $(1+K)$ | log $k\,(1+K)/T$ |
| 30.94 | 0.886 − 4 | 0.000 | 0.228 − 1 |
| 31.93 | 0.476 − 3 | 0.001 | 0.209 − 1 |
| 32.99 | 0.104 − 2 | 0.006 | 0.012 − 1 |
| 34.11 | 0.776 − 2 | 0.025 | 0.629 − 2 |
| 35.32 | 0.494 − 1 | 0.118 | 0.763 − 2 |
| 35.95 | 0.873 − 1 | 0.242 | 0.674 − 2 |
| 36.58 | 0.249 | 0.443 | 0.869 − 2 |
| 37.22 | 0.631 | 0.723 | 0.553 − 2 |
| 37.22 | 0.631 | 0.723 | 0.691 − 2 |
| 37.29 | 0.672 | 0.756 | 0.629 − 2 |

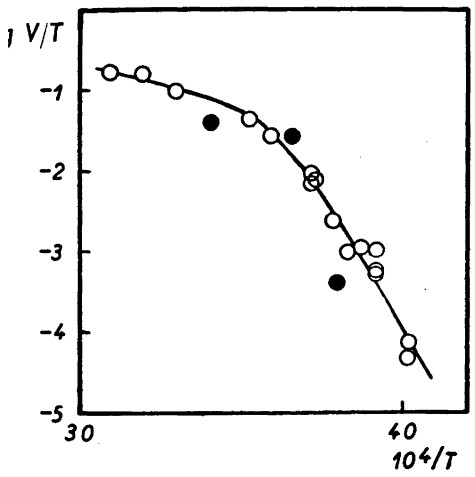


*Fig. 3. The activity of lipase as measured by Sizer and Josephson [13]. The curve was calculated with the constants $\Delta H^* = 4\,700$ cal/mol., $\Delta H = -27\,000$ cal/mole, and $T_{\frac{1}{2}} = 276.4$ °K. Rings: experimental values. Dots: experimental values which due to specified reasons were not considered in calculating the constants.*

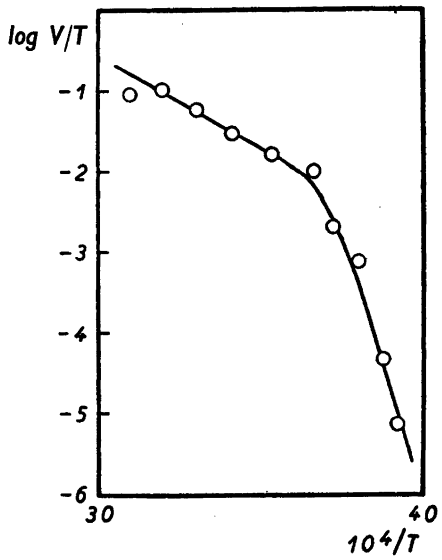


*Fig. 4. The activity of saccharase as measured by Sitzer and Josephson [13]. The curve was calculated with the constants $\Delta H^* = 10\,000$ cal/mole, $\Delta H = -51\,000$ cal/mole, and $T_{\frac{1}{2}} = 269.6$ °K.*

$\Delta H = -27\,000$ cal/mole, and $T_{\frac{1}{2}} = 276.4$ °K fits the experimental results closely.

Sizer and Josephson [13] also investigated the influence of the temperature on the activity of saccharase. It is shown in Fig. 4 that a line with the parameters $\Delta H^* = 10\,000$ cal/mole, $\Delta H = -51\,000$ cal/mole, $T_{\frac{1}{2}} = 269.6$ °K fits the experimental results closely.

Butler [19] measured the specific reaction velocity for the hydrolysis of bensoyl-L-arginine amide with trypsin at 6.0, 15.2, 25.5, and 37.5 °C at pH 7.8, and calculated the enthalpy of activation * on the assumption that a straight line fits the experimental results. Kavanau [17] has shown that a curve fits the experimental points much better than a straight line. Unfortunately, there are not measurements enough to enable an asymptote to be fit to the curve. ** However, we can infer that the enthalpy of activation is probably lower than $\Delta H^* = 13\,800$ cal/mole. This is in agreement with the following. Also Sizer

* Butler's value is $\Delta H^* = 14\,900$ cal/mole. However, the computation is erroneous. He seems first to have computed $\mu = 14\,305$ cal/mole, and then to have added $RT = 543$ cal/mole instead of subtracting this value. Thus, Butler's result should have been $\Delta H^* = 13\,800$ cal/mole.

** See the first foot note on p. 1708.

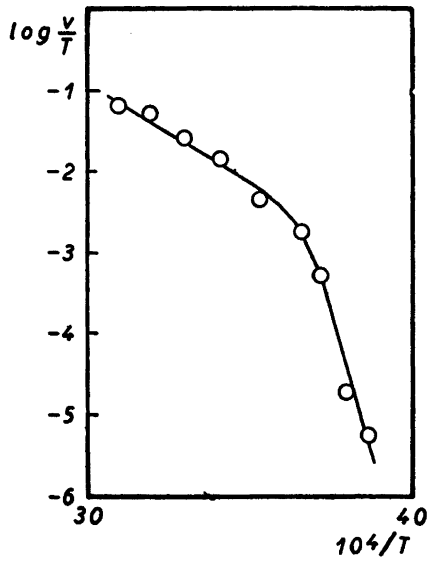


*Fig. 5. The activity of trypsin as measured by Sizer and Josephson [13]. The curve was calculated with the constants* $\Delta H^* = 11\,000$ *cal/mole,* $\Delta H = -53\,000$ *cal/mole, and* $T_{\frac{1}{2}} = 273.1$ *°K.*

and Josephson [13] measured the activity of trypsin at various temperatures. Their experiments were performed at pH 8.1. It is shown in Fig. 5 that a line with the parameters $\Delta H^* = 11\,000$ cal/mole *, $\Delta H = -53\,000$ cal/mole, and $T_{\frac{1}{2}} = 273.1$ °K fits the experimental results closely.

## DISCUSSION

The influence of low temperatures on the activity of enzymes has been interpreted in entirely different ways by Sizer and Josephson [13] and by the present author **. In spite of this, the results of computations of characteristic constants give in both cases two values of enthalpy and one value of a characteristic temperature. The similarities and differences in the results will be discussed here.

In both treatments, one of the fitted lines concerns the same thing, the enthalpy of activation $\Delta H^*$ (or the Arrhenius activation energy $\mu$). The line in Sizer and Josephson's treatment is directly fitted to the experimental points at high temperatures (above the break in the diagram), and in the treatment

* There may also in this case be a reversible heat denaturation for which the values at high temperatures must be corrected before the asymptote can be fitted. Anson and Mirsky [12] found that trypsin, dissolved in 0.01 N HCl is almost completely native at 40 °C but half denatured at 44 °C; however, this temperature of half denaturation was found to be very sensitive to pH and lowered by either acid or alkali.

** See foot note on p. 1702.

suggested here the line is the asymptote fitted to the experimental points. As the asymptote always has less inclination than the line fitted directly to the points, the values calculated according to Sizer and Josephson are higher than the values calculated on the basis of the theory of reversible denaturation.

The other line in the diagram of Sizer and Josephson is also assumed by them to correspond to an energy of activation $\mu$ of the enzymic process, whereas in the treatment given here the other line (Fig. 2) corresponds to the heat of the reversible denaturation of the enzyme.

Finally, the characteristic temperatures have quite different significance: in Sizer and Josephson's treatment it is a temperature at which the kinetics of the enzymic process is suddenly changed — the suggestion of such a sudden change has met several objections [15,17] — whereas in the treatment suggested here the temperature is calculated, at which half of the enzyme is reversibly denatured by the low temperature.

It is worth mentioning here that the experimental errors may by chance turn out so as to suggest the fitting of two or more straight lines in a certain way and also that it would be possible on the basis of Sizer and Josephson's hypothesis to fit straight lines in other ways in several of the published measurements (*cf.* also the corresponding problem, the influence of temperature on biological processes, reviewed by Bělehrádek [14] and discussed by Kavanau [17] and by the present author [20]).

As already mentioned, in most earlier calculations of the energy of activation $\mu$ or the enthalpy of activation $\Delta H^*$ of enzymic processes the results were founded on measurements taken in a comparatively short interval of temperature. It is therefore very likely that several of the results thus obtained are not the true values but are the sum of the true value and part of the negative value of the heat of denaturation.

It has already been mentioned that there is, at least in some cases, a reversible denaturation both at high temperatures and at low temperatures, and that the apparent values of the enzymic activity at various temperatures must first be corrected both for the low temperature and the high temperature denaturation before it is possible to draw the asymptote that corresponds to the true value of the enthalpy of activation.

In the examples of the compution given here it has been assumed that the values of enzymic activity at various temperatures published by Sizer and Josephson [13] are the limit values for high substrate concentrations. If the limit values of the enzymic activity — which are usually denoted $V$ or $k_3$ — are not used, an error is introduced in the computations, due to the temperature dependence of the "Michaelis constant".

It should finally be mentioned that the denaturation both at high and at low temperatures probably depends on the pH [12], which implies that the thermodynamic values computed here are probably valid only for the particular pH at which the experiments were carried out. One other important reservation must also be forwarded: it has been assumed that the amount of native and of denatured enzyme were in equilibrium with each other; however, it is known from Kunitz [21] investigations of the soybean trypsin inhibitor that the high temperature denaturation equilibria are not reached instantly and that the reaction rate depends on the temperature and the pH.

This work was supported by a grant from *Statens Naturvetenskapliga Forskningsråd.* I am indebted to Miss Irma Sjögårdh for valuable assistance.

## REFERENCES

1. van't Hoff, J. H. *Etudes de dynamique chimique*, Frederik Muller & Co., Amsterdam 1884, p. 114.
2. Arrhenius, S. *Z. physik. Chem.* **4** (1889) 226.
3. Eyring, H. *J. Chem. Phys.* **3** (1935) 107.
4. Eyring, H. *Chem. Revs.* **17** (1935) 65.
5. Wassermann, A. *Trans. Faraday Soc.* **34** (1938) 128.
6. Hultin, E. *Acta Chem. Scand. To be published.*
7. Johnson, F. H., Eyring, H. and Polissar, M. J. *The kinetic basis of molecular biology*, John Wiley & Sons, Inc., New York 1954.
8. Eyring, H. and Stearn, A. E. *Chem. Revs.* **24** (1939) 253.
9. Sizer, I. W. *Advances in Enzymol.* **3** (1943) 35.
10. Brown, D. E., Johnson, F. H. and Marsland, D. A. *J. Cellular Comp. Physiol.* **20** (1942) 151.
11. Eyring, H. and Magee, J. L. *J. Cellular Comp. Physiol.* **20** (1942) 169.
12. Anson, M. L. and Mirsky, A. E. *J. Gen. Physiol.* **17** (1934) 393.
13. Sizer, I. W. and Josephson, E. S. *Food Research* **7** (1942) 201.
14. Bělehrádek, J. *Temperature and living matter*, Gebrüder Borntraeger, Berlin 1935.
15. Kistiakowsky, G. B. and Lumry, R. *J. Am. Chem. Soc.* **71** (1949) 2006.
16. Buchanan, R. E. and Fulmer, E. I. *Physiology and biochemistry of bacteria*, Williams & Wilkins Co., Baltimore 1930, Vol. II, p. 47.
17. Kavanau, J. L. *J. Gen. Physiol.* **34** (1950) 193.
18. Snedecor, G. W. *Statistical Methods Applied to Experiments in Agriculture and Biology*, Iowa State College Press, Ames, Iowa 1950.
19. Butler, J. A. V. *J. Am. Chem. Soc.* **63** (1941) 2971.
20. Hultin, E. *Pubbl. staz. zool. Napoli* (1955) 300.
21. Kunitz, M. *J. Gen. Physiol.* **32** (1948) 241.
22. Maier, V. P., Tappel, A. L., and Volman, D. H. *J. Am. Chem. Soc.* **77** (1955) 1278.

Received September 28, 1955.