problem of Dr. Orton's research during the last years, is described with greater details. In the second part of the book dealing with the modern problems relating to oyster culture, the author discusses the ecology of the oyster, its reproduction, methods of obtaining spat, fattening, and the protection of oysters against the attacks of various enemies. The last pages are devoted to the unfavorable environmental conditions, mortality, pollution and purification of oysters. Written in non-technical language, the subject is treated in a scientific manner stressing the idea that fundamental biological research forms the only sound foundation for practical oyster culture. Many references are made to the American oyster, the biology of which is extensively studied in this country. The book is well illustrated with numerous half-tone and line drawings. It is regrettable, however, that for the illustration of the act of spawning of the male and female American oyster the author reproduced a diagram (Fig. 29) which is obviously incorrect. The sperm is always discharged in this species through the cloaca, whereas the illustration shows its emission through the ventro-posterior part of the shell. This oversight does not reflect, however, on the high quality of the book which may be profitably read both by the biologists and those who are interested in the practical aspects of the cultivation of oysters.

There is a selected list of more recent literature and a glossary of terms. Unfortunately there is no index.

PAUL S. GALTSOFF

SPECIAL ARTICLES

THE ENERGY OF ACTIVATION OF PROTEIN DENATURATIONS

IT has been recognized for a quarter century that the kinetics of protein denaturations in general and of enzyme destructions in particular are frequently characterized by exceedingly large temperature coefficients or correspondingly large energies of activation as shown in Table 1.

TABLE 1

Substrate	E (activation)	s = steric factor
Pepsin	64,500	10^{28}
Hemoglobin	60,000	10^{22}
Egg albumin	140,000	10^{72}
Trypsin	150,000	..

With the development in recent years of theories of the absolute rate of reactions,[1] it has become increasingly evident that these reactions are of great interest, since they do not conform to the ordinary laws of chemical kinetics.

The purpose of this paper is to direct attention to the fact that the universally accepted method of calculating the energy of activation may yield fallacious results when applied to reactions which are sensitive to hydrogen ion concentration; that in at least one well-investigated case—the alkaline inactivation of crystalline swine pepsin—the kinetic paradox disappears when it is resolved in terms of a simple mechanism employing definite and familiar concepts.

The velocity constant k may be expressed as

$$k = \nu s e^{-E/RT}. \qquad (1)$$

E is the energy of activation defined through the Arrhenius equation as $RT^2 \left(\frac{\partial \ln k}{\partial T}\right) = E$; the quantities to be held constant in the differentiation will be treated below. The frequency ν may be set equal to Z, the collision number in that variant of the theory[2] or to k T/h in Eyring's more elegant statistical treatment.[3] $Z \simeq 10^{11.4}$ (Sec^{-1}) and $kT/h \simeq 10^{12.8}$ (Sec^{-1}). Values of this order of magnitude are observed empirically for gaseous reactions and for many of the simpler reactions in solutions.[4] Minor deviations from $\nu = Z$ have been ascribed to the steric factor, *s*, to allow for the specific orientations of the molecules necessary for a fruitful collision. For reactions having measurable rates at room temperatures E lies normally between 16,000 and 24,000 cal.

The large values of E quoted above require values of the steric factor which range from 10^{22} to 10^{72}! These astronomical values of *s* at once expose the absurdity of considering these reactions entirely from a collisional point of view.

In this country it has become customary (ref. 2, 3) to express the deviations from normal behavior in terms of the entropy of activation (S) since formally $s = e^{S/R}$. It should be pointed out that although a representation of kinetic abnormalities in terms of an entropy of activation instead of a steric factor has theoretical justification and merit by avoiding the absurdities inherent in the simple collisional theory, an expression of results in terms of entropy of activation *per se* does not constitute an explanation. Only when a definite mechanism is proposed which permits of a numerical comparison of observed and computed entropies of activation can it be claimed that an explanation has been achieved. It should also be men-

[1] The reader is referred to the forthcoming reports of the Symposium on Reaction Kinetics of The Faraday Society (Manchester, September, 1937) for a presentation and critical discussion of these theories.

[2] La Mer, *Jour. Chem. Phys.*, 1: 289, 1933.

[3] Eyring, *Jour. Chem. Phys.*, 3: 107, 1935.

[4] Moelwyn-Hughes, "Kinetics of Reactions in Solutions." Oxford Press, 1933. Chap. IV. Also pp. 50 and 297.

tioned that it is difficult to account for the observed rates by any known process for the redistribution of energy between molecules in gases or in solution when E assumes values as large as those in Table 1.

Stearn and Eyring suggest[5] that the abnormal kinetics of the protein reactions (large entropy of activation) arises from a greater randomness of *intra molecular* motion in the activated state as compared to the original molecule. Support for this view is adduced from Anson and Mirsky's equilibrium data on the entropy increase for the reversible denaturation of trypsin. Eyring has stated[6]: "Thus the mechanism is necessarily one involving the breaking of many weak bonds to form the activated state and any type of mechanism which does not account for this great increase in randomness inside the activated complex is definitely excluded by our theory."

This rather formal view characterized by the vagueness inherent in the expression "intramolecular randomness" gives meager information about the mechanism employed in the process of activation, although highly suggestive correlations have been pointed out.[7,8] The proposal, however, is so intriguing and suggestive that the interpretation of the experimental data merits a critical analysis.

Recently, Jacinto Steinhardt[9] has shown that the rate of destruction of crystalline pepsin, when controlled for salt effects, is accurately proportional to the inverse 5th power of C_{H^+} over a 5,000 fold range of rates. Over an even wider range (18,000 fold) the results are in quantitative agreement with the view, supported by other evidence that the rate is proportional to the concentration of a single species of pepsin ion in which all five of the primary amino groups of the enzyme have undergone acidic dissociation.

The stages of the process may be represented as

$$p^n \overset{K'}{\rightleftarrows} P^{n-5} + 5H^+ \overset{k_1}{\rightleftarrows} X \rightarrow \text{Inactive Product.}$$

P represents the sum of all the ionic species of pepsin exclusive of the five species involved in the reversible acidic dissociation (constant = K′) conditioning the stability. n is the average electric charge of any of the ionic species exclusive of these five. X is the intermediate or critical kinetic complex which finally undergoes irreversible decomposition. k_1 is the velocity constant of the activation process.

The log of the observable velocity constants (k) for 15° and 25° C. are plotted against the p_H in Fig. 1,

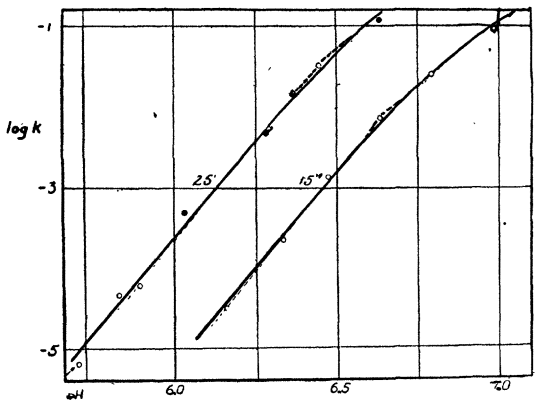

FIG. 1. A comparison of the pH-dependence of the velocity-constant at two temperatures. The curves are from Eq. 3′. The broken straight lines are included as an additional method of appraising the shift in the coordinates of the sets of data, as in Fig. 2.

taken from Fig. 8 of Steinhardt's paper. The points are experimental; the form of curves is computed theoretically from the mechanism postulated above.

When the energy of activation is calculated in the customary manner; namely, by comparing the rates at the same p_H, one obtains (over the linear portion) E = 63,500 cal. However, at least in the present case and presumably in other analogous reactions, this time-honored method of differentiating log k at constant p_H is clearly erroneous. Steinhardt points out that the comparison of rates must be made at equal concentrations of the reactive species P^{n-5} if the calculated E is to represent the energy quantity involved in the activation process (Step II) without complicating this quantity by including an indefinite part of the thermodynamic energy (Δ H) involved in the acidic equilibrium (Step I). The method of separating the contributions from Step I from that of Step II is illustrated in Fig. 2.

Here the points A and B lie on a line representing an arbitrary but constant fraction Y of the primary reactant P^{n-5} to the total pepsin content at the two temperatures T_2 and T_1. The vertical component AC is a measure of the

$$(E)_{p_H} = RT_2T_1 \left(\frac{\Delta \ln k}{\Delta T}\right)_{p_H} = 63{,}500 \text{ cals.}$$

corresponding to Step I + Step II. Whereas the component AD is a measure of

$$(E)_{p_H Y} = RT_2T_1 \left(\frac{\Delta \ln k_1}{\Delta T}\right)_{p_H, Y} = 18{,}300 \text{ cal.}$$

corresponding to Step II. The component DC is a measure of the heat of dissociation of Step I.

$$(\Delta H)_{p_H} = RT_2T_1 \left(\frac{\Delta \ln K'}{\Delta T}\right)_{p_H} = 45{,}200 \text{ cal.}$$

[5] *Jour. Chem. Phys.*, 5: 113, 1937.
[6] Symposium on Reaction Kinetics, ref. 4, Paper No. 4.
[7] M. L. Anson, private communication.
[8] Mirsky and Pauling, *Proc. Nat. Acad. Sci.*, 22: 439, 1936.
[9] *Kgl. Danske Videnskabernes Selskab-Math-Fysik Medd.* (in English), 14: 11, 1937.

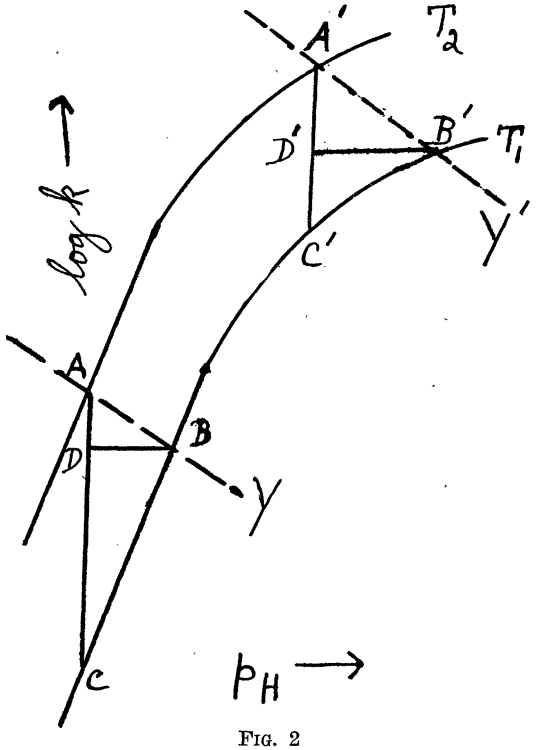

FIG. 2

Steinhardt determines the position of the points A and B (constant Y) by the corresponding coordinates of the theoretical curves when fitted to the experimental points by an appropriate choice of the constants K′ and k. Since Figs. 1 and 2 involve a log *vs.* log plot, this can be accomplished graphically by rectangular transposition. Thus he determines $\triangle p_H$ for constant log k, corresponding to the horizontal component DB, by observing the p_H in the fitted data which the point on the theoretical curve for log K′ − log $a_H = 0$ falls. This p_H is identical with p_K.

Since

$$\triangle p_H = \triangle p_K = \log {}^{K25}/_{K15} = 0.23,$$

$$2.3\, RT_2T_1 \frac{\triangle p_H}{\triangle T} = 9{,}040 \text{ cal.}$$

corresponding to the heat of dissociation of a single proton. Five times this quantity (45,200 cal.) is $\triangle H$ for Step I.

Steinhardt's procedure is thus identical with that given above, which will be valid for the linear portion where practically all the pepsin is present as p^n. The simple procedure of setting AD = AC − DC, however, will not be valid for the less acid non-linear portion, designated by primes in Fig. 2, where a significant fraction of the pepsin exists as P^{n-5}. On the other hand the procedure of subtracting $\triangle$ H, computed from $2.3\, RT_2T_1 \frac{\Delta p_H}{\Delta T}$ is valid for both the linear and the non-linear portions.

When the true energy of activation, 18,300 cal., is employed in eq. 1, the product $\nu s = 10^{9.3}$. Hence *s* lies between $10^{-2.1}$ and $10^{-3.5}$ depending upon whether one assumes $\nu = Z$ or k^T/h. These are reasonable values for the steric factor or true entropy of activation, and the kinetic paradox disappears.

SUMMARY

The abnormally large values of the energy and the entropy of activation encountered in protein denaturations and enzyme destructions are illusory, since the customary method of calculating these quantities by the comparison of rates at constant p_H alone is fallacious. This procedure includes, in addition to the true energy of activation, the heat of dissociation of all acidic equilibria involved in preparing the initial molecules for the kinetic step of activation. Steinhardt has shown by a simple and concrete mechanism that the kinetics of pepsin destruction obey the simple laws of chemical kinetics when correction is made for the preliminary acidic dissociation equilibria which are not part of the activation process.

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NICOTINIC ACID AND VITAMIN B_2

IN collaboration with Dr. Y. Subbarow, of the Department of Biochemistry, Harvard Medical School, I have been attempting to isolate and identify the accessory factors responsible for the prevention of the various diseases which have been attributed to a deficiency of vitamin B_2. Using a crude aqueous extract of liver as raw material, Dr. Subbarow has carried out the chemical fractionations, and biological tests have been made here. Evidence has been obtained which adds one more member to the group of factors comprising vitamin B_2.

Koehn and Elvehjem[1] described a fraction prepared from aqueous extract of liver and showed that it possessed curative properties for chick dermatitis and also for blacktongue in dogs, and concluded from this that the chick dermatitis preventive and blacktongue preventive factors were probably identical. Later, Elvehjem, Madden, Strong and Woolley[2] reported that nicotinic acid cured blacktongue in four dogs. In April of this year we isolated nicotinic acid from a highly purified fraction prepared from liver extract, and later obtained the related alkaloid trigonelline. We had already tested the nicotinic acid for curative activity in chick dermatitis and in rat dermatitis when the publication of Elvehjem *et al.* appeared. When incorporated in the basal diet for chicks described by Lepkovsky and Jukes[3] at levels up to 0.05 per cent. it

[1] *Jour. Biol. Chem.*, 118: 693, 1936.
[2] *Jour. Am. Chem. Soc.*, 58: 1767, 1937.