TEMPERATURE ACTIVATION OF THE UREASE-UREA SYSTEM USING UREASE OF PROTEUS VULGARIS[1]

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The majority of recent temperature studies which have been made on enzyme systems indicate that their rate, like that of chemical reactions, increases exponentially with the absolute temperature in accordance with the Arrhenius equation. With enzymes, however, this temperature activation ceases above the inactivation temperature of the enzyme. For a specific enzyme system the energy of activation (μ in the Arrhenius equation) is constant over a considerable range of temperature; this constancy of μ is characteristic of such systems as the fat oxidation system of *Lupinus albus* (Craig, 1936), yeast and malt invertases (Sizer, 1937), dehydrogenases of *Escherichia coli* (Gould and Sizer, 1938), human and cat bone phosphatase (Bodansky, 1939), jack bean and soy bean urease (Sizer, 1939, 1940), heart dehydrogenase and oxidase (Hadidian and Hoagland, 1939), and purine and aldehyde oxidase of milk (Sizer and Gould, 1940). Constant activation energies characterize intracellular as well as extracellular enzyme systems, and no change in activation energy accompanies the extraction of an enzyme from the cell (Sizer, 1938, 1940).

Information concerning the biochemical relationships of enzymes can be obtained by comparing the activation energies of enzymes from different species. In this respect the invertase

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of yeast (μ = 11,000 cal., Sizer, 1938) differs from that of malt (μ = 13,000 cal., Sizer, 1937), while human and cat phosphatase systems are identical (μ = 9,940 cal., Bodansky, 1939), as are also jack and soy bean urease systems (μ = 8,700 or 11,700 cal., Sizer, 1939, 1940). While it appears that activation energies of corresponding enzymes for closely related species are identical, little information is available for enzymes of distantly related species. The present study of temperature activation of *Proteus vulgaris* urease was undertaken with the purpose of comparing its energy of activation with that of jack and soy bean ureases.

EXPERIMENTAL

Preparation of bacterial suspensions

The organisms used were two strains of *Proteus vulgaris* obtained from the stock culture collection at Brown University. Both strains were motile and fermented maltose, sucrose, glucose, and galactose with the production of acid and gas. Lactose, arabinose, and mannitol were not fermented.

For the preparation of suspensions, eight 250 ml. Erlenmeyer flasks, each containing 125 ml. of the desired sterile medium, were inoculated with 7 to 8 drops of a suspension prepared aseptically from sterile distilled water and a 24-hour nutrient agar slant of the organism. The flasks were incubated at 28° for the desired time. At the end of the incubation period the organisms were centrifuged and then resuspended in distilled water to a satisfactory dilution (usually to 30 ml.). Rewashing of the organisms with distilled water, saline or dilute nutrient broth yielded unsatisfactory suspensions with unusually low urease activity. Controls for respiratory activity were run. Suspensions were refrigerated when not in use. The concentration of organisms in each suspension was determined by total nitrogen estimations on an aliquot by a modification of the Koch-McMeekin (1924) micro-Kjeldahl method.

Measurement of urease activity

A stock solution was prepared which contained 3 per cent urea, 5.4 per cent Na_2HPO_4, and 4.25 per cent KH_2PO_4. The

phosphate buffered the digest to pH 7.0 (optimum pH for urease activity) and kept the alkalinity produced by the liberated NH_3 from increasing by more than 0.1 pH unit while the reaction was being studied. To 2 ml. of urea-phosphate solution were added 1 ml. of bacterial suspension and 1 ml. of stabilizer which was usually an oxidizing or a reducing solution. The stabilizer was added in view of the fact that the urease molecule is unstable and can be readily activated or inactivated by the addition of reducing or oxidizing agents (Hellerman, 1937). The activation energy of the jack bean urease-urea system is 11,700 cal. when oxidizing agents are present, but is 8,700 cal. in the presence of neutral or reducing agents (Sizer, 1939). Similarly, in the case of soy bean urease (Sizer, 1940) stabilizers are important in determining whether the activation energy will be 8,700 or 11,700 cal. With every bacterial preparation, four experiments were performed at each temperature, using H_2O alone, or 0.2 M KCN, 0.01 M Na_2SO_3, or 0.2 M $Na_2S_2O_3$ as stabilizers.

During the course of urea hydrolysis the liberated NH_3 dissolves in the solution, while the CO_2 is evolved and can be measured manometrically (Van Slyke, 1927; Krebs and Henseleit, 1932; Sizer, 1939, 1940). Hydrolysis follows the same apparent course whether studied by measurement of NH_3 production colorimetrically after nesslerization or CO_2 evolution manometrically with the Barcroft differential manometer (Sizer, 1939).

The 4 ml. of reaction mixture were placed in one cup of the manometer and 4 ml. of water in the control cup. From 2 to 3 minutes adaptation to the temperature of the water bath were allowed before the stop-cocks were closed. Usually ten manometer readings were taken at each temperature during the time required for the evolution of 100 to 500 c.mm. of gas. The temperature of the water bath was controlled to $\pm$ 0.05°. Gas volumes were converted to the standard temperature of 0° in order to make them comparable.

RESULTS

In figure 1 is plotted CO_2 evolution as a function of time for a digest containing 2 ml. urea-phosphate, 1 ml. water and 1 ml.

bacterial suspension (culture grown 18 hours at 28° in a 1 per cent tryptone, 0.2 per cent urea, 1 per cent glycerol medium). From the figure it is apparent that the reaction follows a linear course during the first phase of the urea hydrolysis at all temperatures between 0.2 and 50°. Similar kinetics of hydrolysis have been reported for jack bean and soy bean ureases (Sizer, 1939, 1940). Rates of hydrolysis were calculated from the slopes of the straight lines drawn through the plotted points and expressed as c.mm. CO_2 evolved per minute. This proved to be an

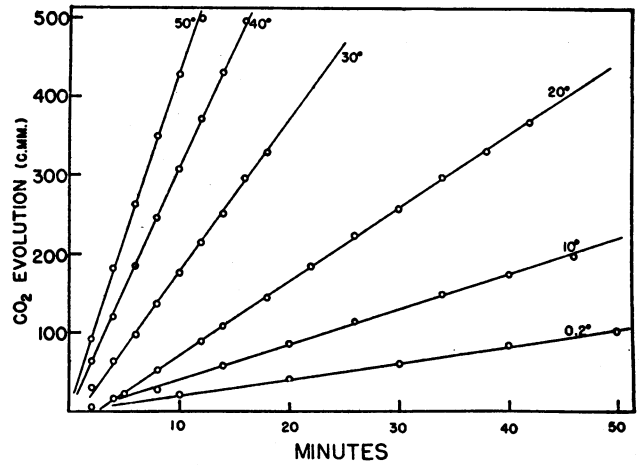

FIG. 1. Hydrolysis (as measured by c.mm. of CO_2 evolved) of 1.5 per cent urea (in phosphate buffer, pH 7.0) by *Proteus vulgaris* (grown 18 hours in a medium containing 1 per cent tryptone, 0.2 per cent urea and 1 per cent glycerol); urease is plotted as a function of elapsed time in minutes for several different temperatures.

accurate method of measuring rate since errors of single readings are rendered unimportant. While the data of figure 1 are typical, there were occasional bacterial preparations which were so unstable that the plotted points fell off from a straight line after only 200–300 c.mm. CO_2 had been evolved. With a few preparations at 0.2° the reaction followed a curvilinear course for a brief period before it became linear. In all cases, however, it was possible to calculate rate of hydrolysis from the linear portion of the curve. The linearity of the curves was in general independent

of the presence of stabilizers, although the urease activity was sometimes slightly inhibited by KCN.

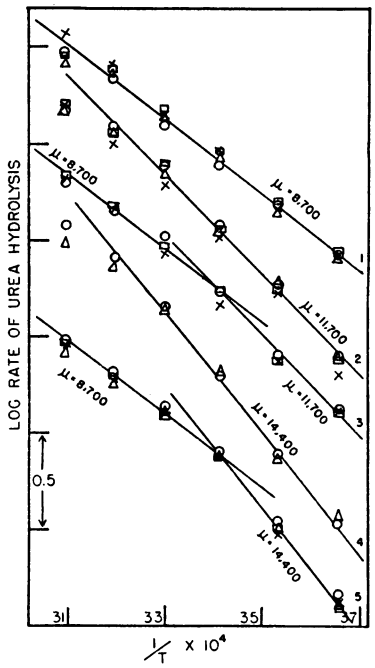

FIG. 2. LOG RATE OF UREA HYDROLYSIS (CO_2 EVOLVED PER MINUTE) BY PROTEUS VULGARIS UREASE PLOTTED AGAINST THE RECIPROCAL OF ABSOLUTE TEMPERATURE

The distribution of the curves along the ordinate is arbitrary. In each experiment the digest contained 2 ml. urea-phosphate, 1 ml. bacterial suspension, and 1 ml. of one of the following: ○, H_2O; X, 0.2 M KCN; □, 0.01 M Na_2SO_3; △, 0.2 M $Na_2S_2O_3$.

1. 19 hour culture grown in medium containing 1 per cent peptone and 1 per cent glucose.
2. 18 hour culture grown in medium containing 1 per cent tryptone, 0.2 per cent urea, and 1 per cent glycerol.
3. 23 hour culture grown in medium containing 1 per cent peptone, 0.2 per cent urea, and 0.03 per cent tyrosine.
4. 12 hour culture grown in medium containing 1 per cent peptone, 0.2 per cent urea, and 1 per cent glycerol.
5. 20 hour culture grown in medium containing 1 per cent peptone, 0.2 per cent urea, and 1 per cent mannitol.

In all 360 runs which have been made over the temperature range from 0.2 to 50° the data have been analyzed by determining rates of CO_2 evolution at each temperature. Log rate was then plotted against the reciprocal of the absolute temperature (fig. 2).

Since in all cases the plotted points are best fitted by a straight line or by two straight lines intersecting at a critical temperature, it is apparent that the data are in accord with the Arrhenius equation

$$\mu = \frac{4.58 \log (k_2/k_1)}{1/T_1 - 1/T_2}$$

where μ is the energy of activation in calories per gm. mole of the urease-urea system, and k_1 and k_2 the rates at the corresponding absolute temperatures, T_1 and T_2. At 40° or 50° the points fall off from the straight line due to heat inactivation of the enzyme.

The activation energies of the urease-urea system calculated from the slopes of the lines in the 360 Arrhenius plots which were made of the data, were always either 8,700, 11,700, or 14,400 cal. regardless of the condition of growth or the composition of the digest. One of these three values may characterize a given urease system over the whole temperature range (fig. 2, curves 1, 2 and 4) or there may be two activation energies, one representing the activation energy of the system below, the other the activation energy above a critical temperature (fig. 2, curves 3 and 5). In the case of a break in the Arrhenius curve the activation energy is always 11,700 or 14,400 cal. below and 8,700 cal. above the critical temperature (fig. 2, curves 3 and 5). The five possible temperature relationships are presented in figure 2. A given urease preparation will yield data consistent with one or more of these five possible categories, depending on the composition of the nutrient medium, the presence or absence of a carbon source (other than peptone, etc.) or of urea in the growth medium, the age of the culture, the growth temperature, and the nature of the stabilizer added to the digest.

Relationship of activation energy of the urease system to growth conditions of Proteus vulgaris

1. Nutrient medium. When the organism is grown for 17 to 72 hours in a medium containing only peptone, or tryptone, or nutrient broth, or proteose-peptone the activation energy is

TABLE 1

Activation energies and enzyme activity of the urease-urea system of Proteus vulgaris as functions of the composition of the culture medium and length of growth period

CULTURE MEDIUM*	CULTURE AGE	ACTIVATION ENERGY	N PER ML. SUSPENSION	UREASE ACTIVITY C.MM. CO_2 PER MIN.	UREASE ACTIVITY PER MGM. BACTERIAL N
	hours	*cal.*	*mgm.*		
Peptone	21	11,700–8,700†	.5	6.89	13.8
Peptone	48	11,700–8,700	.6	2.80	4.65
Nutrient broth	20	11,700–8,700	.4	3.24	5.31
		8,700			
Tryptone	17	8,700	1.2	2.91	2.52
Tryptone	48	8,700	1.8	5.99	3.32
		11,700			
Tryptone	72	8,700	.63	2.82	4.49
		11,700			
Proteose-peptone	19.5	11,700–8,700	.5	2.10	4.20
		8,700			
Proteose-peptone	23	8,700	.45	2.16	4.79
Proteose-peptone	41	8,700	.95	4.20	4.42
Peptone, glucose	19	8,700	.5	2.32	4.65
Peptone, glycerol	48	11,700–8,700	2.7	6.90	2.55
Tryptone, glucose	19	11,700–8,700	.9	4.85	5.37
		8,700			
Peptone, urea	19	14,400–8,700	.65	10.3	15.9
Peptone, urea	43	14,400–8,700	.6	11.5	19.2
Peptone, urea	72	8,700		4.92	
Tryptone, urea	19	14,400–8,700	.8	17.0	21.3
Tryptone, urea	48	11,700–8,700	.6	12.5	20.8
		8,700			
Nutrient broth, urea	24	11,700–8,700	.6	15.8	21.6
Proteose-peptone, urea	3.75	14,400	.25	3.14	12.5
		11,700			
Proteose-peptone, urea	6	14,400–8,700	.5	9.71	22.2
		11,700–8,700			
Proteose-peptone, urea	13	11,700–8,700	1.0	14.0	14.0
Proteose-peptone, urea	24	11,700–8,700	.4	13.5	33.6
Tryptone, urea, glycerol	12	14,400–8,700	.4	19.9	4.96
Tryptone, urea, glycerol	18	11,700	1.7	18.6	11.0
Tryptone, urea, glycerol	44	8,700	3.8	6.75	1.77
		11,700–8,700			
Tryptone, urea, glucose	20	14,400–8,700	3.2	6.66	18.0
Tryptone, urea, glucose	24	14,400–8,700	4.0	3.80	9.51
Proteose-peptone, urea, glucose	22	14,400–8,700	5.4	9.30	17.2

* Substances added to the culture medium had the following concentrations: peptone 1 per cent, nutrient broth 0.8 per cent, tryptone 1 per cent, proteose-peptone 1 per cent, glucose 1 per cent, glycerol 1 per cent, urea 0.2 per cent.

† I.e., 11,700 cal. below and 8,700 cal. above the critical temperature.

always either 8,700 or 11,700 cal. over the whole temperature range, or 11,700 cal. below and 8,700 cal. above a critical temperature (cf. table 1). When *Proteus* is grown under these conditions the effects of temperature upon its urease system are identical with those upon the urease system of jack or soy beans.

2. Nutrient medium plus glucose or glycerol. Cultures grown for 19 to 48 hours in a nutrient medium to which had been added glucose or glycerol contained urease whose activation energy was the same as that of cultures grown in the unmodified nutrient medium (table 1).

3. Nutrient medium plus urea. The addition of urea to the nutrient medium profoundly modifies the growth and metabolism of the organism when grown for 3.75 to 72 hours. Although activation energies of 8,700 or 11,700 cal. still characterize the urease of many cultures, a new value of 14,400 cal. is associated with many other cultures. The value of 14,400 cal. may characterize the system over the whole temperature range (fig. 2, curve 4) or may change to 8,700 cal. at the higher temperatures (fig. 2, curve 5). The data of table 1 suggest that as the culture (grown in a nutrient medium-urea solution) ages the activation energy may change from 14,400 to 11,700 cal. The effect of age on activation energy is discussed on page 521.

4. Nutrient medium plus urea and a carbon source. Essentially no change was produced in the activation energy of the urease-urea system by growing the organism in tryptone-urea-glycerol instead of tryptone-urea, or in proteose-peptone-urea-glucose instead of proteose-peptone-urea (table 1), although the addition of the carbon source produced a tremendous increase in growth.

An extensive study has been made of the activation energy of the urease system as influenced by the nature of the carbon source added to the peptone-urea growth medium of *Proteus* (table 2). In the case of all carbon sources except lactose and nitrogen-containing compounds the activation energy of an 18 to 24 hour culture is 14,400 cal. below and 8,700 cal. above the critical temperature. The addition of sugars, alcohols, and salts of organic acids to the peptone-urea growth medium do not modify the activation energy of the urease system.

TABLE 2

Activation energies and enzyme activity of the urease-urea system of Proteus vulgaris (grown in 1 per cent peptone, 0.2 per cent urea) as functions of the composition of the culture medium

ADDED CARBON SOURCE*	CULTURE AGE	ACTIVATION ENERGY	N PER ML. SUSPENSION	UREASE ACTIVITY C.MM. CO_2 PER MIN.	UREASE ACTIVITY PER MGM. BACTERIAL N
	hours	*cal.*	*mgm.*		
Sucrose	20	14,400–8,700	8.6	56.0	6.46
Glucose	20	14,400–8,700	3.4	34.7	10.2
Glucose	45	14,400–8,700 8,700	8.0	37.6	4.73
Galactose	18	14,400–8,700	6.8	30.2	4.45
Galactose	48	11,700–8,700	7.2	42.9	4.49
Fructose	22	14,400–8,700 11,700–8,700	2.0	30.8	15.4
Fructose	48	11,700–8,700	3.9	16.8	4.28
Maltose	45	11,700–8,700 8,700	1.9	9.10	4.79
Lactose	22	11,700–8,700		12.4	
Lactose	45	11,700–8,700 8,700	1.6	13.8	8.59
Xylose	44	11,700–8,700	1.3	9.21	7.18
Mannitol	20	14,400–8,700	1.4	11.5	8.25
Mannitol	44	11,700–8,700	.9	6.14	6.80
Inositol	45	11,700–8,700	1.0	7.36	7.36
Acetate†	20	14,400–8,700 11,700–8,700	.2	10.5	5.26
Acetate†	48	14,400–8,700 11,700–8,700	.67	14.5	21.6
Lactate	20	14,400–8,700	1.0	9.72	9.72
Succinate	44	11,700–8,700 8,700	1.3	11.3	8.69
Tartrate	40	14,400–8,700	1.0	7.00	7.00
Tartrate	72	11,700–8,700	.35	4.03	11.5
Asparagine	14	11,700–8,700 8,700	.9	20.2	22.5
Asparagine	24	11,700–8,700	1.9	8.04	4.22
Asparagine	44	8,700	1.8	16.7	9.29
Tryptophane, 600 mgm	22	11,700–8,700	.7	12.9	18.4
Tryptophane, 300 mgm	22	11,700–8,700	.4	11.0	27.5

* Except where indicated the concentration of the carbon source was 10 grams per liter of culture medium.

† Sodium salts of the acids were used.

TABLE 2—*Concluded*

ADDED CARBON SOURCE*	CULTURE AGE	ACTIVATION ENERGY	N PER ML. SUSPENSION	UREASE ACTIVITY C.MM. CO_2 PER MIN.	UREASE ACTIVITY PER MGM. BACTERIAL N
	hours	*cal.*	*mgm.*		
Tryptophane, 100 mgm.....	22	14,400–8,700	.8	15.2	19.0
Cysteine, 300 mgm.....	20	11,700–8,700	.35	12.7	36.1
Cystine, 300 mgm.....	24	11,700–8,700 8,700	1.0	13.7	13.7
Glycine, 300 mgm.....	21	11,700–8,700	.45	24.2	53.8
Tyrosine, 300 mgm.....	23	11,700–8,700	.6	11.7	19.4
Tyrosine, 300 mgm.; glucose, 300 mgm.....	24	14,400–8,700	2.0	70.0	3.50
Nicotinic acid, 20 mgm.....	20	8,700	.55	8.29	15.1
Nicotinic acid, 20 mgm.....	23	11,700–8,700 11,700 8,700		7.42	
Nicotinic acid, 5 mgm.....	21	14,400–8,700	.4	8.31	20.8

On the other hand, lactose (not fermented by this strain of *Proteus*) amino acids and nicotinic acid, when added to the peptone-urea growth medium, so modified the metabolism that the activation energy of the urease system of an 18 to 24 hour culture is either 8,700 or 11,700 cal. Evidently the influence of urea in the growth medium in favoring an activation energy of 14,400 cal. for the urease system has been completely neutralized by the addition of lactose or a nitrogen-containing carbon source to the peptone-urea growth medium, with the result that the μ of 14,400 cal. is replaced by a μ of 11,700 cal. In other words, the activation energies are the same as for a culture grown in the absence of urea (table 1). If the concentration of the nitrogen-containing carbon source in the peptone-urea growth medium is decreased to a critical point it ceases to have any influence on the activation energy of a 20 hour bacterial preparation. For example, with 600 or 300 mgm. tryptophane per liter, or 20 mgm. nicotinic acid per liter added to the growth medium the activation energies are 11,700 and 8,700 cal., but with only 100 mgm. tryptophane or 5 mgm. nicotinic acid they are 14,400 and 8,700 cal.

Relationship of activation energy of the urease system to age of bacterial culture

In table 3 are presented data showing the relationship between the age of a culture (grown in peptone-urea-glycerol culture medium) and the activation energy of the urease system. From the table it is clear that urease cultures 24 hours old or younger are characterized by activation energies of 14,400 or 8,700 cal., while for cultures 52 hours old or older the corresponding values are 11,700 or 8,700 cal. Any of the three values may be obtained using cultures grown for 40 to 48 hours. It thus appears that as the culture ages the activation energy of 14,400 cal. abruptly changes to 11,700 cal., while the value of 8,700 cal. does not change. The change from μ = 14,400 cal. to μ = 11,700 cal., with aging of the culture is characteristic not only of *Proteus* cultures when grown in a peptone-urea-glycerol medium, but also when grown in tryptone-urea, proteose-peptone-urea, tryptone-urea-glycerol, peptone-urea-galactose, peptone-urea-fructose, peptone-urea-mannitol, and peptone-urea-tartrate (cf. tables 1 and 2). In all of these cases there has been a transition from μ = 14,400 to 11,700 cal. for the urease system as the incubation period of the bacteria is increased. It is also apparent that for these cultures a μ of 8,700 cal. may characterize the urease of both young and old cultures.

Since the transition from μ = 14,400 to 11,700 cal. for the urease system as the culture ages is probably correlated with growth and metabolism it would be expected that an acceleration or retardation of these factors might cause the transition in activation energy to occur either earlier or later in the growth period. This problem was attacked by growing the organism at different temperatures, and it was found that such was in general the case; in cultures grown at 37° the transition occurred earlier in the growth period, while in cultures grown at 10° or 15° it occurred later, as compared with the usual cultures grown at 28°. It is interesting to note that even though the organism is incubated at temperatures unfavorable for growth, none the

less, the same three activation energies characterize the urease as when the organism is grown at an optimum temperature.

Relationship of activation energy of the urease system to the strain of Proteus vulgaris employed

While different strains of a bacterial species may vary considerably in their biochemical and morphological characteristics, the same activation energies are found for the urease system of *Proteus vulgaris* strain no. 2 as for no. 1 which has been regularly employed. Strain no. 2 was grown for 24 hours in a tryptone-urea and in a tryptone-urea-glucose medium. The corresponding activation energies were 11,700 cal. below and 8,700 cal. above 18° for the former, while for the latter culture they were 14,400 cal. below and 8,700 cal. above 28°. From these results it is apparent that the activation energies of the urease system are the same for these two different strains of *Proteus vulgaris*.

Relationship of activation energy to the stabilizer added to the digest

A change from one to another of the three activation energies can be effected not only by varying the conditions under which the bacteria are grown but also by changing the composition of the digest by the addition of H_2O or a stabilizer[2] such as, 0.2 M KCN, 0.01 M Na_2SO_3, or 0.2 M $Na_2S_2O_3$. For example, in the case of a 20 hr. nutrient broth culture (table 1) the activation energy was 8,700 cal. when KCN was present in the digest, while with H_2O, Na_2SO_3, or $Na_2S_2O_3$ present it was 11,700 cal. below and 8.700 cal. above 25°. Similarly, a 48 hr. peptone-urea-glycerol culture (table 3) was characterized by activation energies of 14,400 and 8,700 cal. with H_2O or $Na_2S_2O_3$, by 11,700 and 8,700 cal. with Na_2SO_3, and by 8,700 cal. with KCN as stabilizer. An analysis of all experiments indicated that KCN favors to some degree an activation energy of 8,700 cal., but no other correlation was noted. Despite the fact that the activation energy of certain urease preparations could be changed from one of the three typical values to another, in two-thirds of the experiments

[2] In a few experiments the stabilizers 0.2 M $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, H_2O_2, $KClO_4$, and $FeCl_2$ were also employed with similar results.

the urease was so stable that the activation energy could not be changed by the use of different stabilizers.

Urease activity and cell concentration as functions of various growth conditions

The total nitrogen of a sample of an aqueous suspension of the organisms after centrifugation was determined and was taken as a measure of the number of cells present (Wilson, 1938). The urease activity of a digest containing 2 ml. urea-phosphate, 1 ml.

TABLE 3

Activation energies and enzyme activity of the urease-urea system of Proteus vulgaris (grown in 1 per cent peptone, 1 per cent glycerol, 0.2 per cent urea) as functions of the growth period of the culture

CULTURE AGE	ACTIVATION ENERGY	N PER ML. SUSPENSION	UREASE ACTIVITY C.MM. CO_2 PER MIN.	UREASE ACTIVITY PER MGM. BACTERIAL N
hours	*cal.*	*mgm.*		
6	14,400–8,700		1.74	
12	14,400–8,700 14,400	1.0	13.6	13.6
24	14,400–8,700	1.9	11.2	5.87
40	14,400 11,700	2.5	19.9	7.94
43	14,400–8,700 14,400	1.6	11.0	6.89
48	14,400–8,700 11,700–8,700 8,700	1.4	9.31	7.91
52	11,700–8,700	.54	6.89	12.7
72	11,700–8,700	2.1	7.91	3.78

H_2O, and 1 ml. bacterial suspension was measured at 20° and expressed as total activity and activity per milligram of nitrogen of the bacterial suspension (tables 1, 2, and 3).

From the data it appears that only moderate crops were obtained in a culture containing a single nutrient, or a nutrient plus urea, or glucose, or glycerol. When either glucose, galactose, fructose, or sucrose was added to a nutrient medium containing urea, however, the crop was tremendously increased. All the carbon sources other than these four sugars, when added to a

peptone-urea medium (table 2), induced little or no stimulation of growth. In general, the greater the concentration of cells the greater the total urease activity of the bacterial suspension. The urease activity per milligram N (a measure of the urease concentration per bacterial cell) is not correlated with growth. The addition of urea to a nutrient medium doubles or trebles the urease activity per milligram N. (cf. table 1). If a carbon source contains N (amino acids or nicotinic acid) however, the urease per milligram N is greatly increased. It thus appears that urea and other nitrogen-containing compounds can stimulate the synthesis of urease by *Proteus vulgaris*. This is especially interesting in view of the fact that these same compounds, when added to the culture medium, are especially effective in determining whether the activation energy of the urease-urea system shall be 14,400 or 11,700 cal. Similar stimulation of bacterial enzyme synthesis by the addition of various substances to the growth medium has been reported by other workers (cf. Anderson, 1938).

DISCUSSION

In the few experiments where duplicate runs were made at all six temperatures the rates of reaction did not differ from the average by more than 5 per cent. In most experiments four separate studies were made on a single bacterial culture at each temperature. Since the activities and energies of activation were the same for the four stabilizers in $\frac{2}{3}$ of the experiments, there are quadruplicate checks of urease activity at each temperature (cf. fig. 2). The straight lines drawn through the plotted points were fitted by eye and the corresponding activation energies are accurate to about $\pm$ 300 cal.

Of the three activation energies which characterize the urease system of *Proteus vulgaris* it seems most significant that two of these, namely 8,700 and 11,700 cal., are identical with those which characterize the urease of the jack bean, *Canavalia ensiformis*, and the soy bean, *Glycine hispida*. For all three ureases under certain conditions the activation energy may be 8,700 or 11,700 cal. over the whole temperature range or 11,700 cal.

below and 8,700 cal. above a critical temperature. For all three ureases a shift in activation energy can be effected by changing the composition of the digest. In addition, the activation energy of *Proteus vulgaris* urease can be modified by altering the metabolism of the organism, e.g., by changing the composition of the culture medium or by changing the duration of the growth period. The activation energy of 14,400 cal. for *Proteus vulgaris* urease under certain conditions has not been obtained with the bean ureases. In view of the interconvertibility of the three activation energies of *Proteus vulgaris* it seems most probable that they characterize a single urease molecule which can be activated in three different ways, rather than three different kinds of urease.

The fact that two of the activation energies of *Proteus vulgaris* urease are identical with those of jack bean and soy bean ureases is considered strong evidence for the close similarity of a single enzyme obtained from widely different species of organisms. This identity of activation energy of similar enzymes from diverse species offers an explanation for the fact that in different organisms the temperature characteristics are the same for many physiological processes (Crozier, 1924; Hoagland, 1936) where the rate is presumably controlled by the underlying enzyme-catalyzed reactions.

SUMMARY

The kinetics of urea hydrolysis as a function of temperature have been studied using the intracellular urease of *Proteus vulgaris*. The course of the reaction was followed by measuring CO_2 evolution with the Barcroft manometer. At all temperatures CO_2 evolution is a linear function of time. Rate of hydrolysis was calculated from the slope of the straight line drawn through the plotted points.

Over the temperature range from 0.2 to 40° or 50° the data are in accord with the Arrhenius equation where the energy of activation is 14,400, 11,700, or 8,700 cal. per gram mole, depending upon the metabolism of the organism, the age of the culture and the composition of the urea digest. With certain

bacterial preparations the activation energy was 14,400 or 11,700 cal. below and 8,700 cal. above a critical temperature.

The value of 8,700 cal. was often obtained under all conditions, the value 11,700 cal. was characteristic of those cultures grown in the absence of urea, or old cultures grown in the presence of urea. The value of 14,400 calories never characterized the urease of a culture which had been grown in the absence of urea, but was typical of the urease of young cultures grown with urea present in the growth medium. The addition of water alone or certain stabilizers such as KCN, Na_2SO_3, or $Na_2S_2O_3$, to the urease-urea digest in the majority of cases had no effect, although in some instances the use of different stabilizers caused the activation energy to change from one to another of these three values.

The urease concentration per bacterial cell is differentially stimulated by the addition to the growth medium of urea, amino acids, or nicotinic acid.

The fact that the activation energies of 11,700 and 8,700 cal. for the *Proteus* urease system are identical with those of the urease system of jack and soy beans suggests a close similarity in the ureases of these widely different species.

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