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12

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The Optimum Temperature of Salicin Hydrolysis by Enzyme Action is Independent of the Concentrations of Substrate and Enzyme.

By ARTHUR COMPTON, B.A., M.B., R.U.I., Imperial Cancer Research Fund.

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The object of the present investigation is to ascertain the influence, if any, on the optimum temperature—temperature of greatest activity—of an enzyme, of the concentration, on the one hand, of the substrate, and, on the other, of the enzyme. The investigation, involving two variables, presents three cases for consideration, according as the concentration of the substrate and the concentration of the enzyme are varied separately or together. An account is given of the results obtained with the enzyme or enzymic function, present in sweet-almond emulsin, which hydrolyses the glucoside salicin with the production of equimolecular quantities of glucose and saligenin. A commercial specimen of Merck's emulsin was used, while the purity of the salicin employed was ascertained by determining its melting point (200·5°) and its optical activity ($[\alpha]_D^{21} = -62{\cdot}7°$).

The successive stages in the inquiry may be briefly summarised as follows: (1) a preliminary determination of the activity of the specimen under certain chosen conditions as regards the concentration of the substrate, the temperature, and the duration of the experiment; (2) a preliminary determination of the optimum temperature with the quantity of enzyme found capable of producing 50 per cent. hydrolysis of the substrate under the above conditions; (3) a determination of the activity curves of the enzyme at the temperature thus found, in an action of the same duration for five concentrations of the substrate M/5, M/10, M/15, M/30, and M/50; (4) a determination of the optimum temperature of the enzyme for each of the five concentrations of the substrate in presence of a constant enzyme concentration; (5) a determination of the optimum temperature of the enzyme for each of the five concentrations of the substrate with quantities of enzyme indicated by the activity curves as capable of producing 70 per cent. hydrolysis of the substrate in the given time: (6) a determination of the optimum temperature of the enzyme for a constant concentration of the substrate in presence of different enzyme concentrations.

The preliminary determination of the activity of the enzyme was carried out in a M/5 dilution of the substrate during a period of 15 hours at 40°. The practical details were as follows: 286 mgrm. of salicin and varying quantities of the enzyme dissolved in 5 cm.^{3} of water, specially purified by redistillation under diminished pressure, were introduced into each of a series of seven clean Jena glass test-tubes. The tubes were incubated for 15 hours in a water thermostat at 40°, after which the enzyme action was stopped by rapidly cooling the tubes and then adding to each a drop of concentrated solution of ammonium hydroxide. The proportion of glucoside hydrolysed in each tube was estimated by the increase of reducing power, measured by the method of Bertrand.* The numbers obtained are set out in Table I.

Table I.

Quantity of enzyme.	Salicin hydrolysed.
mgrm.	per cent.
0·6	34·8
1·5	67·2
3·0	86·8
4·5	91·2
6·0	91·7
7·0	96·1
9·0	97·0

* 'Bull. Soc. Chim.' [3], 1906, vol. 35, p. 1285.

If the percentages of salicin hydrolysed be plotted as ordinates and the quantities of enzyme as abscissæ these numbers give the activity curve shown in fig. 1.

The preliminary determination of the optimum temperature of the enzyme, the second stage of the inquiry, was carried out as follows: A solution of the enzyme was prepared containing 10 times the quantity of

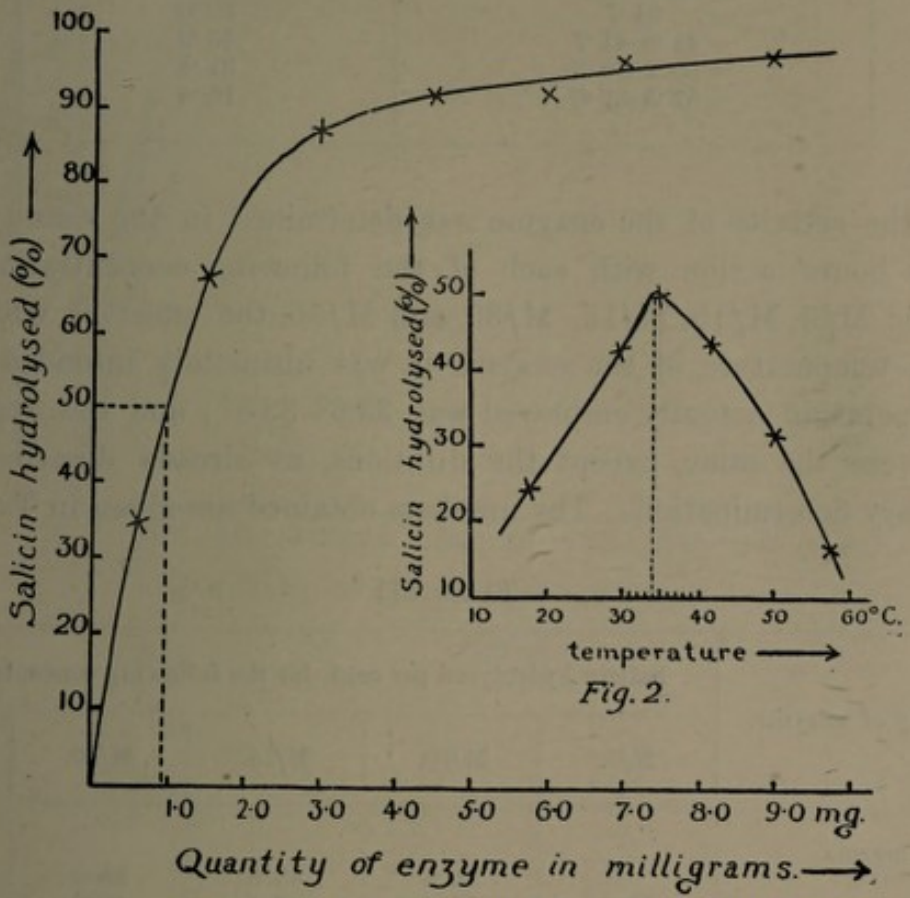

Fig. 2.

Fig. 1.

enzyme necessary to produce the percentage of hydrolysis decided upon—in this case 0·9 mgrm. for a 50 per cent. hydrolysis as shown by fig. 1—dissolved in 10 cm.^{3} of redistilled water. After half to one hour of contact at the ordinary temperature the solution was introduced in portions of 1 cm.^{3} into each of a series of eight or nine test-tubes already containing 286 mgrm. of salicin and 4 cm.^{3} of water. The tubes were then plunged into water-baths kept at known temperatures, and after 15 hours the action was stopped and the proportion of glucoside hydrolysed determined as before. The numbers obtained are set forth in Table II.

By plotting the percentage of salicin hydrolysed against the mean temperature of the experiment these numbers give the curve indicated above in fig. 2. The optimum temperature under the foregoing conditions is thus found to be +34°.

Table II.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed.
°	per cent.
17·8–17·6	24·2
29·3–29·5	42·8
34·7	50·0
41·8–41·7	43·0
50·2–50·3	31·4
57·5–57·6	16·4

Next the activity of the enzyme was determined in the vicinity of +34° for a 15 hours' action with each of the following concentrations of the substrate: M/5, M/10, M/15, M/30, and M/50, the effect of which on the optimum temperature of the enzyme it was ultimately intended to study. The temperature actually employed was 33·6°–33·8°; and the experimental details were the same, except the dilutions, as already described for the preliminary determination. The numbers obtained are given in Table III.

Table III.

Quantity of enzyme.	Salicin hydrolysed per cent. for the following concentrations:—				
	M/5.	M/10.	M/15.	M/30.	M/50.
mgrm.					
0·5	—	31·4	35·8	28·2	—
1·0	55·1	57·5	58·5	49·5	36·9
2·0	79·4	84·7	88·7	77·0	—
3·0	88·3	93·9	97·5	90·0	77·2
5·0	94·2	97·5	99·6	99·0	93·9
7·0	94·9	96·7	99·2	100·6	100·0
10·0	95·7	100·2	99·6	—	100·5
12·5	95·7	—	100·6	100·6	100·0

These numbers give, on plotting the percentage of salicin hydrolysed against the quantity of enzyme in play, the activity curves shown in fig. 3.

The influence of the substrate concentration on the optimum temperature of the ferment, the fourth stage of the inquiry, may now be considered. This is the case of determining the optimum temperature in a series of experiments in which the concentration of the enzyme is kept constant while that of the substrate varies. The concentration of the enzyme chosen, in accordance with fig. 3, was 0·4 mgrm. in 5 cm.^{3}, *i.e.* 8×10^{-5} grm. per cm.^{3} of the reaction mixture. Five different solutions of the enzyme were prepared

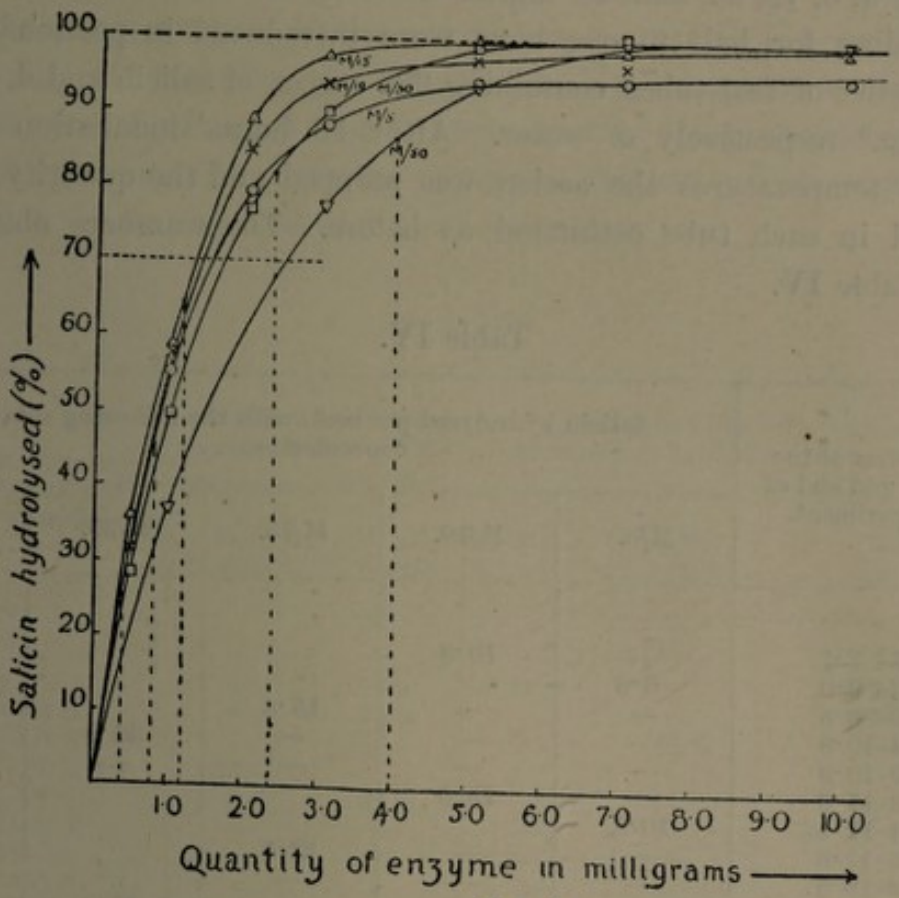

Fig. 3.

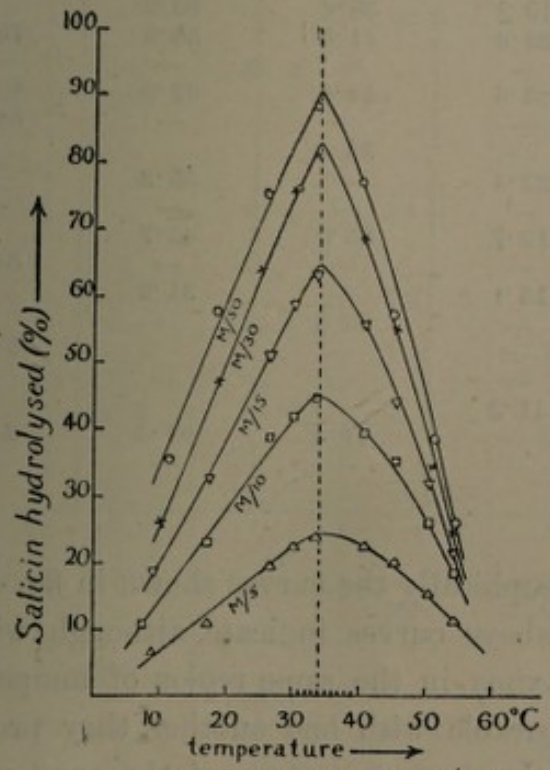

Fig. 4.—{Substrate conc^{n} M/5 to M/50
Enzyme conc^{n} 8×10^{-5} gr. per cm^{3}.

containing 4, 8, 12, 24, and 40 mgrm. dissolved in 10 cm.^{3} of water, which, after standing for half to one hour, were introduced in portions of 1 cm.^{3} into five series of test-tubes containing 286 mgrm. of salicin and 4, 9, 14, 29, and 49 cm.^{3} respectively of water. After 15 hours' incubation in baths at known temperatures the action was stopped and the quantity of salicin hydrolysed in each tube estimated as before. The numbers obtained are given in Table IV.

Table IV.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following substrate concentrations :—				
	M/5.	M/10.	M/15.	M/30.	M/50.
°					
7·3– 7·7	—	10·8			
8·3– 9·0	6·6				
9·6– 8·5	—	—	18·3		
9·4–10·8	—	—	—	26·0	
12·0–10·9	—	—	—	—	35·5
17·2–17·3	—	23·0			
17·4–17·1	10·8				
17·5–17·6	—	—	32·5		
18·6–18·5	—	—	—	—	57·5
18·9	—	—	—	47·0	
25·2–25·0	—	—	—	63·7	
26·4–25·7	—	—	—	—	75·0
26·5	19·2	38·6	50·8		
30·0	22·3	41·9	58·5	75·3	
30·5	—	—	—	—	75·8
33·5	23·6	44·6	62·9	80·7	88·3
40·5	—	—	—	68·3	76·7
40·7–40·6	—	39·4			
40·8–40·7	22·4	—	55·2		
45·0–45·2	—	—	—	—	56·9
45·5–45·3	19·7	35·0	43·7		
45·5	—	—	—	54·7	
50·3–50·5	15·1	—	31·9		
50·6–50·5	—	25·8	—		
51·1–50·8	—	—	—	34·3	
51·5–51·2	—	—	—	—	38·3
54·3–54·0	11·2				
54·2–54·3	—	18·4	21·4	24·3	
54·6–55·0	—	—	—	—	25·8

These numbers give graphically the curves shown in fig. 4 (p. 249).

On examination the above curves indicate, although with very different degrees of precision, maxima in the same region of temperature. In so far as the curves are comparable with one another, they produce the general impression that the optimum temperature of the enzyme is constant, and consequently independent of the concentration of the substrate. But to answer the question more definitely curves of a uniform type, easily com-

parable among each other, are required. This can only be achieved by varying the concentration of the enzyme at the same time as that of the substrate, for, *ceteris paribus*, the extent of an enzyme action is, as shown by the activity curves of figs. 1 and 3, determined by the proportion of enzyme to substrate present in the reaction mixture. The case which constitutes the fifth stage of the inquiry will now be considered.

Table V.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following molecular concentrations of the substrate and grm. of the enzyme per cm.^{3}.					
	M/5. 31×10^{-5}.	M/10. $13{\cdot}5 \times 10^{-5}$.	M/15. $8{\cdot}7 \times 10^{-5}$.	M/30. $5{\cdot}7 \times 10^{-5}$.	M/50. 5×10^{-5}.	
°						
8·0	—	—	17·2			
9·0- 8·3	—	17·3				
9·5- 9·0	—	—	—	18·8		
17·8–17·5	35·0					
18·1–18·2	—	34·2				
18·2–18·4	—	—	—	—	39·7	
18·4	—	—	—	—	—	41·9
18·7–18·5	—	—	—	40·3		
18·8–18·7	—	—	36·3			
23·1–22·5	46·1					
24·9–25·1	—	—	—	—	50·5	
26·1–26·2	—	—	53·3			
26·2–26·4	—	—	—	50·3	—	61·1
27·0	—	56·9				
29·7	62·2					
30·0–30·5	—	—	—	65·7		
30·5	—	—	—	—	68·6	69·4
31·8–31·6	—	—	63·7			
33·5–33·8	—	69·4	65·8			
33·8	—	—	—	70·0	—	70·0
34·7–34·8	—	—	—	—	71·7	
35·6–35·7	—	—	65·8			
36·0	—	69·4				
36·6	—	—	—	66·3		
39·4–39·9	—	—	—	—	59·7	
41·8–42·0	60·5	58·3				
42·5–43·0	—	—	—	56·3		
43·0	—	—	—	—	—	53·0
43·6–43·5	—	—	51·2			
44·0–44·4	—	—	—	—	47·0	
47·8–48·0	—	43·3				
48·0–48·5	—	—	—	37·7		
48·5	—	—	37·7	—	—	36·4
49·0–49·5	—	—	—	—	34·1	
50·2	44·6					
51·0	—	35·6				
51·7–51·5	—	—	30·7			
53·5	—	—	—	22·7		
53·5-53·8	—	—	—	—	22·5	22·4
54·6	33·6					
57·5–57·4	23·7					

That the curves might be vertical enough to give sharply defined maximum points, it was decided to aim at obtaining about 70 per cent. hydrolysis of the substrate at the optimum temperature. A cursory examination of fig. 3, which was constructed at approximately +34°, shows that to obtain such curves—assuming for the moment, what fig. 4 already indicates the probability of, that the optimum temperature is independent of the concentration of the substrate, and situated at about + 34°—the quantities of enzyme required, in actions of 15 hours' duration, are 1·55, 1·35, 1·30, 1·70 and 2·50 mgrm. respectively for the concentrations M/5, M/10, M/15, M/30 and M/50 of the substrate. Working with these quantities the experimental data obtained are set forth in Table V.

By plotting as before the percentage of salicin hydrolysed against the temperature of the experiment the foregoing numbers give the curves represented in figs. 5, 6, 7, 8 and 9.

On careful examination the curves below all show that the activity of the enzyme is greatest between +33·5° and +34·5°; in other words, that the optimum temperature is about +34°, and is constant, notwithstanding the wide variations in the dilution of the substrate and the accompanying variations in the dilution of the enzyme.

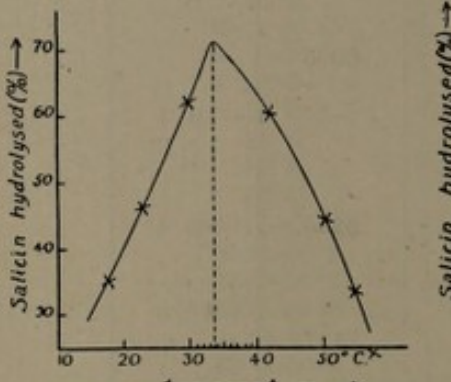

Fig. 5.—{Substrate conc^n M/5. Enzyme conc^n $3{\cdot}1\times10^{-5}$ gr. per cm^3

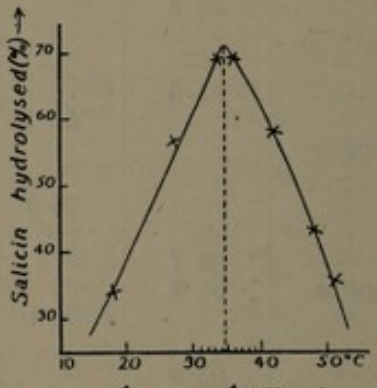

Fig. 6.—{Substrate conc^n M/10. Enzyme conc^n $13{\cdot}5\times10^{-5}$ gr. per cm^3.

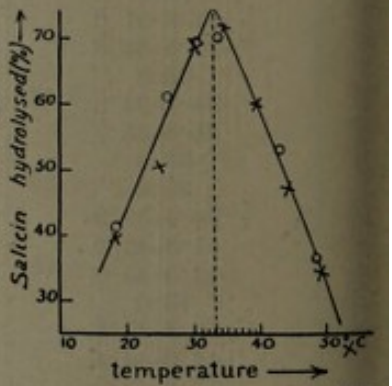

Fig. 9.—{Substrate conc^n M/50. Enzyme conc^n 5×10^{-5} gr. p

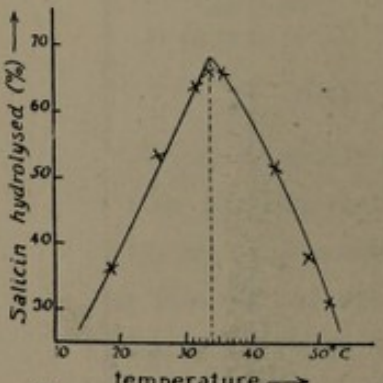

Fig. 7.—{Substrate conc^n M/15 Enzyme conc^n $8{\cdot}7\times10^{-5}$ gr. per cm^3.

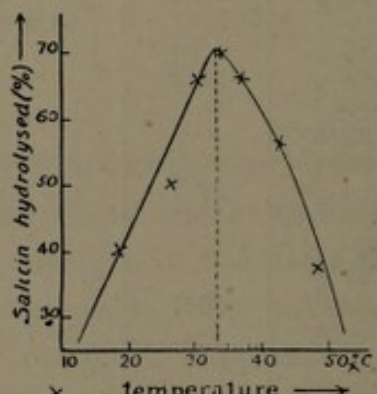

Fig. 8.—{Substrate conc^n M/30 Enzyme conc^n $5{\cdot}7\times10^{-5}$ gr. per cm^3.

Table VI.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following enzyme concentrations per cm.^{3}.					
	$1{\cdot}8 \times 10^{-5}$.	$3{\cdot}3 \times 10^{-5}$.	$6{\cdot}8 \times 10^{-5}$.	$11{\cdot}7 \times 10^{-5}$.	15×10^{-5}.	18×10^{-5}.
°						
8·5– 8·3	—	—	—	—	43·7	
9·5– 8·5	—	—	—	—	—	48·7
10·0–10·5	—	16·0				
17·5	—	—	—	—	70·0	
17·8–17·9	—	24·7				
18·2–18·0	—	—	—	—	—	78·7
18·7–18·6	—	—	—	63·0		
19·0	18·3					
19·5–19·0	—	—	47·7			
20·3–20·1	—	—	—	—	—	84·0
21·5–21·1	—	—	51·6			
22·5	—	—	—	—	—	92·0
24·4	—	—	—	78·0		
24·8–24·9	—	—	61·7			
25·5–25·6	22·5					
25·8–25·6	—	38·0				
26·2	—	—	—	—	90·0	
30·0	—	—	—	—	95·3	
30·2	25·2	46·8				
30·4–31·6	—	—	—	91·0		
31·1	—	—	—	—	—	99·0
31·6–31·7	—	—	76·0			
32·9–33·1	—	—	—	94·7		
33·5	—	46·8	—	—	98·0	
34·0	25·2					
37·6–37·8	—	—	74·2			
40·0–39·9	—	—	—	—	—	99·7
40·2	23·0					
40·5	—	43·3	—	85·0		
40·7	—	—	—	—	99·0	
41·8–42·0	—	—	65·3			
45·0–45·2	—	35·8	—	73·3	84·0	
45·0–45·4	18·8					
49·1–49·0	13·5					
50·5–50·4	—	—	—	—	61·3	69·0
51·1–51·0	—	—	—	47·7		
52·2–51·6	—	—	—	—	—	60·7
53·6–53·5	—	—	26·8			
54·0	—	14·6	—	—	42·7	
54·3	—	—	—	35·0		
55·0	—	—	—	—	—	44·3
56·0	6·5					
57·0	—	—	18·0			

Turning now to the last stage of the inquiry, the case of the substrate concentration remaining constant while that of the enzyme changes, it constitutes the study, properly speaking, of the influence of the enzyme concentration on the optimum temperature. Although rendered unnecessary by what precedes, the study is given in order to complete the present investigation. For this a M/30 dilution of the substrate was chosen and

the optimum temperature determined in actions of 15 hours' duration with quantities of the enzymic specimen giving concentrations varying between $1{\cdot}8 \times 10^{-5}$ and 18×10^{-5} grm. per cm.^{3}. The numbers obtained are given in Table VI. The results are recorded graphically in fig. 10.

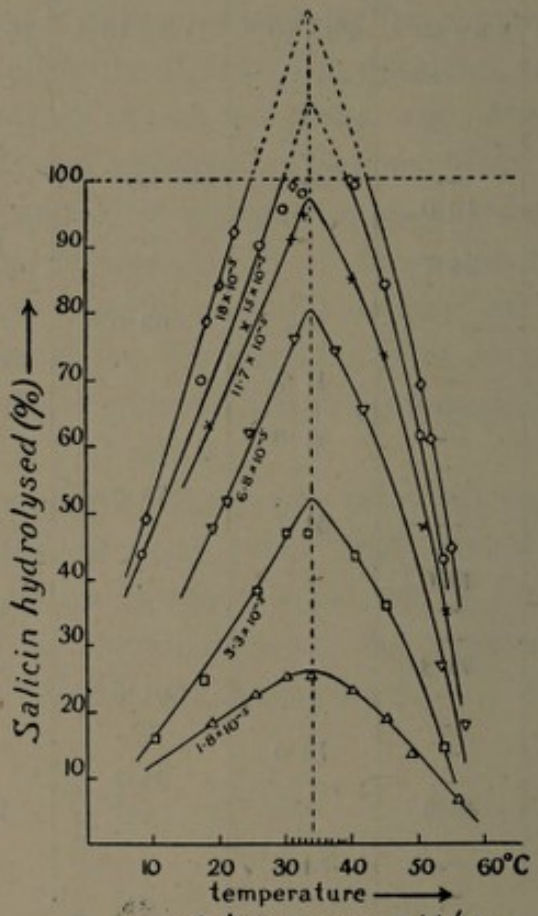

Fig. 10.—{ Substrate concⁿ M/30.
Enzyme concⁿ $1{\cdot}8 \times 10^{-5}$ to 18×10^{-5} gr. per cm.^{3}

The curves of fig. 10, as well as the M/30 curve of fig. 4 and that of fig. 8, show that the optimum temperature of the enzyme is the same in each, and, consequently, independent of the concentration of the enzyme. This holds true, as shown by two of the curves in fig. 10, even when the proportion of enzyme to substrate is more than sufficient to produce complete hydrolysis of the substrate at the optimum point. Here the optimum point is imaginary, and corresponds to the intersection of the curves representing respectively the activation and the destruction of the enzyme by heat.

Briefly, then, the outcome of the inquiry is, for an action of known duration, the optimum temperature of the enzyme investigated is independent alike of the concentration of the substrate and of the concentration of the enzyme. Whether the statement be true of enzymes in general—as theoretical considerations would lead one to expect—I propose to answer by fresh experiments on other types of enzymes.

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