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*Constancy of the Optimum Temperature of an Enzyme under
Varying Concentrations of Substrate and of Enzyme.*

By ARTHUR COMPTON.



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Constancy of the Optimum Temperature of an Enzyme under Varying Concentrations of Substrate and of Enzyme.

By ARTHUR COMPTON, Imperial Cancer Research Fund.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received June 10,—Read June 25, 1914.)

In a recent paper* a new enzymic relation is recorded. For the enzymic hydrolysis of salicin—by the enzyme which Gabriel Bertrand and the author† have named *salicinase*—it is found that, in an action of fixed duration,‡ the temperature of greatest activity of the ferment is always the same, whatever the dilutions of substrate and of enzyme adopted for the determination. In other words, the duration of the action being constant, the optimum temperature of the ferment is independent of the concentration both of the substrate and of the enzyme. The observation is suggestive: if true of one enzyme it may be true of all, and possibly becomes the enunciation of a general law. Herein, for the moment, lies its main interest.

* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 87, p. 245 (1914).

† Gabriel Bertrand and A. Compton, 'Comptes Rendus,' vol. 157, p. 797 (1913).

‡ For the variation of the optimum temperature of an enzyme with the duration of the enzyme action, see Gabriel Bertrand and A. Compton, 'Comptes Rendus,' vol. 152, p. 1518 (1911); 'Ann. Inst. Past.,' vol. 26, p. 161 (1912).

In the present paper further experimental evidence for this hypothesis is given, in the case of another hydrolytic enzyme, the *maltase* of *Aspergillus oryzae* (taka-diastase).

For the extract of *Aspergillus oryzae* used, the Imperial Cancer Research Fund is indebted to Messrs. Parke, Davis and Co., who placed at my disposal one of their most active preparations. This preparation, after being freed from insoluble constituents and purified by a technique to be detailed elsewhere, consists of a white powder, entirely soluble in water, whose activity in *maltase* is double that of the original preparation.

The maltose used was Kahlbaum's. It was purified by successive recrystallisations from water, the mother liquor impurities being removed after each recrystallisation by pressing the crystals in an hydraulic press between several layers of clean dry linen. Eventually, after powdering in a mortar and drying for about a week *in vacuo* over sulphuric acid, a specimen of pure maltose, containing one molecule of water of crystallisation, was obtained. It gave an optical activity $[\alpha]_D^{20} = +130.4^\circ$, and its reducing power, determined by Bertrand's method,* was as set out in Table I.

Table I.

Weight of maltose.	Weight of copper.
mgram.	mgram.
20.0	21.0
40.0	42.0
60.0	62.0
80.0	83.0
100.0	103.5

These numbers, allowing for the molecule of water of crystallisation present, correspond exactly with those given by Bertrand (*ibid.*).

That the optimum temperature of the ferment is independent of the concentration of the substrate is shown by the following experiments:—Four series of eight clean Jena glass test-tubes were prepared containing respectively 360, 180, 90, and 60 mgram. of maltose dissolved in 4 cm.³ of water which had been specially purified by redistillation under diminished pressure. Then into each tube was introduced in portions of 1 cm.³ a solution of the enzyme, prepared a half to one hour previously, containing 10 mgram. per cm.³. The substrate concentrations in the four series of tubes are M/5, M/10, M/20, and M/30. The tubes, after being closed with clean sterile corks, were plunged into water-baths kept at known temperatures. After

* 'Bull. Soc. Chim.,' (3), vol. 35, p. 1285 (1906).

16 hours' incubation the tubes were withdrawn, the corks removed, and each rapidly washed with 1 cm.³ of water, the washings being carefully added to the contents of the corresponding tube. The tubes were next heated for five minutes in boiling water to stop the enzyme action, they were then cooled, and the contents of each diluted to a known volume, such that 20 cm.³ of the diluted mixture corresponds to 36 mgrm. of maltose. The proportion of maltose hydrolysed was estimated by the increase of reducing power as determined by the method of Bertrand (*ibid.*). The numbers obtained are recorded in Table II.

If the percentage of maltose hydrolysed be plotted against the mean temperature of the experiment these numbers give the series of curves represented by fig. 1.

Each curve shows a maximum at or about the same point, +47°. Hence, under the conditions of the experiment, the optimum temperature of the ferment is constant, and independent of the variations in the concentration of the substrate.

That the optimum temperature is also independent of the concentration of the enzyme is shown by the following experiments:—Four solutions of the enzyme were prepared containing 10, 30, 60 and 100 mgrm. dissolved in 10 cm.³.

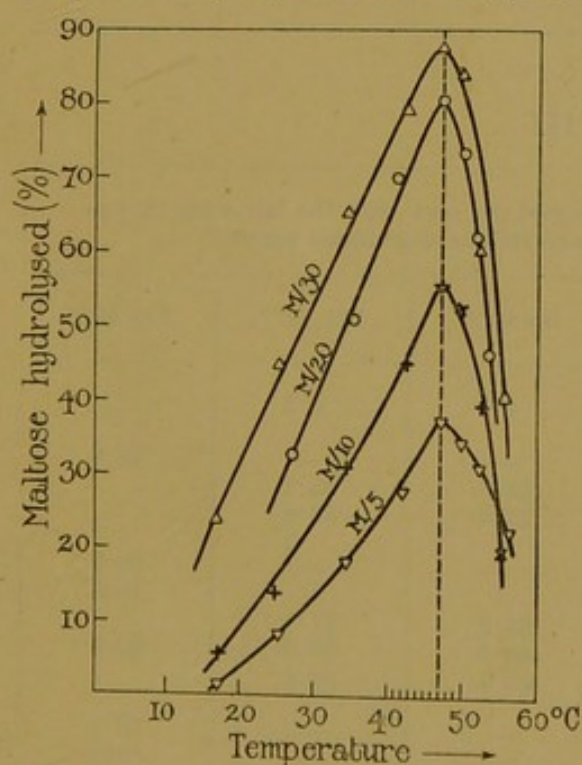


FIG. 1.

FIG. 1.—Substrate concentrations M/5 to M/30. Enzyme concentration 2×10^{-3} gm. per cm.³

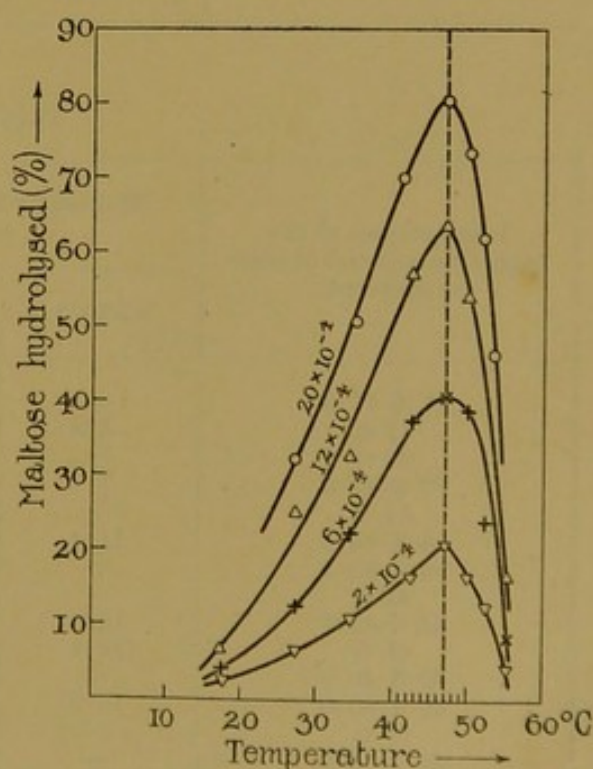


FIG. 2.

FIG. 2.—Substrate concentration M/20. Enzyme concentrations 2×10^{-4} to 20×10^{-4} gm. per cm.³

Table II.

Temperatures at the beginning and end of each experiment.	Maltose hydrolysed per cent. with the following substrate concentrations.			
	M/5.	M/10.	M/20.	M/30.
17.0	1.9	—	—	24.0
17.2-17.0	—	5.9	—	—
25.0	—	14.2	—	—
25.3-25.5	8.6	—	—	45.0
27.0	—	—	32.8	—
34.3-34.4	—	31.2	—	—
34.5	18.3	—	—	65.2
35.0-35.1	—	—	50.9	—
41.0	—	—	70.0	—
42.4	28.2	—	—	78.5
42.5-42.4	—	45.0	—	—
47.0	37.5	55.6	80.3	87.3
49.5-49.6	—	52.5	—	—
49.5-50.0	34.3	—	—	83.8
50.0	—	—	73.4	—
51.5-52.0	—	—	61.9	—
52.2	31.2	—	—	60.3
52.2-52.4	—	39.1	—	—
53.3-53.2	—	—	46.4	—
54.5-55.2	—	19.7	—	—
55.5-55.4	22.5	—	—	40.6

Table III.

Temperatures at the beginning and end of each experiment.	Maltose hydrolysed per cent. with the following enzyme concentrations in grammes per cm. ³			
	2×10^{-4} .	6×10^{-4} .	12×10^{-4} .	20×10^{-4} .
17.3-17.5	—	4.5	7.2	—
17.6	3.2	—	—	—
27.0	—	—	—	32.8
27.3-27.5	7.2	12.8	25.4	—
34.3	—	22.5	32.8	—
34.5-34.4	11.4	—	—	—
35.0-35.1	—	—	—	50.9
41.0	—	—	—	70.0
42.4-42.5	16.9	35.9	57.1	—
47.0	21.1	40.6	63.5	80.3
49.8-49.9	—	39.1	54.1	—
49.7-50.0	16.9	—	—	—
50.0	—	—	—	73.4
51.5-52.0	—	—	—	61.9
52.1-52.2	—	24.0	—	—
52.3-52.4	12.8	—	—	—
53.3-53.2	—	—	—	46.4
55.2-55.0	4.5	—	—	—
55.2	—	8.6	16.9	—

of water, which, after standing from a half to one hour, were introduced in portions of 1 cm.³ into four series of test-tubes containing 90 mgrm. of maltose dissolved in 4 cm.³ of water. The concentration of the substrate in this experiment is M/20, while the enzyme concentration varies between 2×10^{-4} and 20×10^{-4} gm. per cm.³. After 16 hours' incubation, the action was stopped, and the quantity of maltose hydrolysed in each tube was determined as before. The numbers obtained are set out in Table III.

On plotting the percentage of maltose hydrolysed against the mean temperature of the experiment the curves of fig. 2 are obtained.

Here, again, each curve shows a maximum in the same region of temperature, +47°. Consequently, the optimum temperature of the enzyme is independent of the enzyme concentration.

Thus it is found, for the *maltase* of *Aspergillus oryzae*—as for the *salicinase* of sweet almonds—that the optimum temperature of the ferment is independent alike of the concentration of the substrate and of the concentration of the enzyme.

