XIII. ON THE OPTIMAL CONDITIONS FOR THE PROTEOCLASTIC ACTION OF TAKA-DIASTASE.

By SEIZABURO OKADA (Tokio).

From the Institute of Physiology, University College, London.

(Received January 19th, 1916.)

Introduction. Taka-diastrase is prepared from koji-yeast and is chiefly used for the artificial digestion of amylaceous food. Koji-yeast itself is produced by the influence of *Aspergillus oryzae* on soaked rice. *A. oryzae* produces various kinds of ferments. Atkinson [1881] was the first to note that koji contains a strong diastase. Kellner, Mori and Nagaoka [1890] found that it converts starch into maltose and dextrin and, finally, into grape sugar; moreover, that it converts cane sugar into invert sugar. Saito [1906] reported that *A. oryzae* produces also trypsin, rennet, and catalase. Wohlgemuth [1912] found also erepsin, lipase and a haemolytic ferment. The conditions of the diastatic action of koji-yeast or taka-diastrase have been studied by various authors. Kellner, Mori and Nagaoka observed that lactic acid above 0.1 % weakens the action of koji-diastrase, but in a concentration of 0.05 % accelerates its action somewhat. They found also that it is strongly influenced in its diastatic or inverting action by the presence of salt, but does not completely lose its action even with 20 % of salt content. Saito also found this influence of salt in the case of pure cultures of the organism. According to Leo [1896], taka-diastrase is not so sensitive to acids as is ptyalin, while Strauss and Stargard [1898] found no difference between them. Wohlgemuth confirmed Leo and even found the addition of dilute hydrochloric acid to accelerate the action of taka-diastrase. With regard to its proteoclastic function, Wohlgemuth found on the contrary that taka-diastrase is more active in media which are neutral or very slightly alkaline than in those which are very slightly acid.

CONDITIONS OF PROTEOCLASTIC ACTION OF TAKA-DIASTASE 131

The clinical use of taka-diastrase was studied by Leo, and by Strauss and Stargard. These authors found that its amyloclastic function is alone of importance. So far as my experience goes, taka-diastrase is very effective also in the digestion of proteins. This fact is not surprising, since Wohlgemuth found that 1 g. taka-diastrase contains as much proteoclastic ferment as 100 cc. of human or animal pancreatic juice. This proteoclastic function seems more important than the amyloclastic and is of more clinical value, since the digestion of proteins is more frequently defective than that of carbohydrates. Consequently it is desirable to study the optimal conditions in which this ferment acts. As the proteoclastic ferment contained in taka-diastrase is very similar in its action to that of pancreatic juice, I applied the method which Michaelis and Davidsohn [1911] used to study trypsin, with some modification in details. As ferment I used commercial taka-diastrase which was always freshly dissolved to 10 % concentration. This solution, if kept 24 hours or longer, becomes black in colour, so that titration is interfered with, though it does not lose its activity. As substrate Witte's peptone was used, dissolved to 4 % in distilled water by boiling. It was filtered and kept sterile. The conversion was determined by Sørensen's formal method [1908]. For each experiment there were taken 10 cc. of regulator solution mixed with 30 cc. of 4 % peptone solution and, after warming to the required temperature, 10 cc. of 10 % taka-diastrase solution were added. 10 cc. of digestion mixture were taken immediately after mixing and afterwards at any desired time, were put into a beaker containing 10 cc. of 40 % formal to stop digestion and then titrated with N/10 NaOH. Ten drops of 1 % phenolphthalein solution were used and a difference of less than 0.1 cc. could be detected, as Michaelis and Davidsohn observed with trypsin. The temperature was kept between 37–38° and several comparative estimations were made at the same time to control each other. The reaction of the digestion mixtures was measured by the electrometric method, which was carried out at the temperature of the room, mentioned in each case. Following the method employed by Michaelis and Davidsohn in their research on trypsin, I added such a quantity of regulators (mostly phosphate or acetate mixtures) as to neutralise the influence of the products of digestion on the reaction. Even in 24 hours no noticeable change occurred. The results are given in Table I.

From Table I it is to be seen that the optimum for the action of taka-diastrase on Witte's peptone is at $[H] = 8.5 \times 10^{-6}$. This is shown also graphically in the figure.

With regard to the resistance of taka-diastrase to acids Strauss and Stargard

TABLE I.

No.	Regulator mixture	[H']	P _H	Time mins.	N/10 NaOH formal titration cc.
1	5.0 cc. N/3 Na ₂ HPO ₄ ...	9.1 × 10 ⁻¹²	11.04 (19° C.)	20	0.10
	3.0 cc. N/1 NaOH ...			40	0.28
	2.0 cc. distilled water ...			60	0.39
2	5.0 cc. N/3 Na ₂ HPO ₄ ...	8.5 × 10 ⁻⁹	8.07 (17.5° C.)	20	0.94
	1.5 cc. N/1 NaOH ...			40	1.74
	3.5 cc. distilled water ...			60	2.34
3	5.0 cc. N/3 Na ₂ HPO ₄ ...	8.9 × 10 ⁻⁹	8.05 (19° C.)	20	1.08
	1.0 cc. N/1 NaOH ...			40	1.80
	4.0 cc. distilled water ...			60	2.40
4	5.0 cc. N/3 Na ₂ HPO ₄ ...	4.2 × 10 ⁻⁸	7.38 (17.5° C.)	20	1.34
	0.5 cc. N/1 NaOH ...			40	2.02
	4.5 cc. distilled water ...			60	2.72
5	0.7 cc. N/3 NaH ₂ PO ₄ ...	9.1 × 10 ⁻⁸	7.04 (19° C.)	20	1.42
	7.0 cc. N/3 Na ₂ HPO ₄ ...			40	2.25
	2.3 cc. distilled water ...			60	3.10
6	Same as 5, but with boiled ferment ...			20	0
				40	0
				60	0
7	4.0 cc. N/3 NaH ₂ PO ₄ ...	4.0 × 10 ⁻⁷	6.40 (16.5° C.)	20	1.62
	4.0 cc. N/3 Na ₂ HPO ₄ ...			40	2.53
	2.0 cc. distilled water ...			60	3.35
8	4.5 cc. N/3 NaH ₂ PO ₄ ...	4.1 × 10 ⁻⁷	6.39 (16.5° C.)	20	1.62
	4.0 cc. N/3 Na ₂ HPO ₄ ...			40	2.65
	1.5 cc. distilled water ...			60	3.40
9	7.0 cc. N/3 NaH ₂ PO ₄ ...	1.1 × 10 ⁻⁶	5.96 (17.5° C.)	20	1.67
	3.0 cc. distilled water ...			40	2.65
				60	3.40
10	1.0 cc. N/1 acetic acid ...	2.1 × 10 ⁻⁶	5.68 (19.0° C.)	20	1.70
	3.0 cc. N/1 sodium acetate ...			40	2.70
	6.0 cc. distilled water ...			60	3.50
11	1.5 cc. N/1 acetic acid ...	4.3 × 10 ⁻⁶	5.37 (17.0° C.)	20	1.75
	2.5 cc. N/1 sodium acetate ...			40	2.80
	6.0 cc. distilled water ...			60	3.60
12	2.0 cc. N/1 acetic acid ...	8.5 × 10 ⁻⁶	5.07 (17° C.)	20	1.73
	2.0 cc. N/1 sodium acetate ...			40	2.90
	6.0 cc. distilled water ...			60	3.65
13	Same as 12 ...			20	1.78
				40	2.90
				60	3.67
14	Same as 12 ...			20	1.75
				40	2.85
				60	3.65
15	Same as 12 ...			20	1.84
				40	2.82
				60	3.62
16	Same as 12, but with boiled ferment ...			20	0
				40	0
				60	0

CONDITIONS OF PROTEOCLASTIC ACTION OF TAKA-DIASTASE 133

TABLE I (*continued*).

No.	Regulator mixture	[H ⁺]	P _H	Time mins.	N/10 NaOH formal titration cc.
17	2.5 cc. N/1 acetic acid ...	1.6 × 10 ⁻⁵	4.80 (20° C.)	20	1.62
	1.5 cc. N/1 sodium acetate			40	2.56
	6.0 cc. distilled water ...			60	3.53
18	3.0 cc. N/1 acetic acid ...	2.6 × 10 ⁻⁵	4.59 (16° C.)	20	1.50
	0.3 cc. N/1 sodium acetate			40	2.30
	6.7 cc. distilled water ...			60	2.86
19	5.0 cc. N/3 NaH ₂ PO ₄ ...	1.9 × 10 ⁻⁴	3.72 (15° C.)	20	0.95
	2.0 cc. N/1 HCl ...			40	1.61
	3.0 cc. distilled water ...			60	2.05
20	Same as 19, but with boiled ferment ...			20	0
				40	0
				60	0
21	5.0 cc. N/3 NaH ₂ PO ₄ ...	1.4 × 10 ⁻²	1.84 (17° C.)	20	0.19
	5.0 cc. N/1 HCl ...			40	0.24
				60	0.29

found that the enzyme after one hour's contact with hydrochloric acid or gastric juice, of which the acidity is over 30 (0.11 %), loses entirely its amyloclastic power, so that no regeneration of its action in the intestine is to be expected when the medium resumes its neutral reaction. In respect to its proteoclastic function, my results are as follows:

Exp. 1. 1 g. taka-diasatase was dissolved in 5.0 cc. N/10 HCl + 5.0 cc. N/10 NaOH. As regulator mixture was used 2.0 cc. acetic acid + 2.0 cc. sodium acetate + 6.0 cc. distilled water. Digestion was carried out in the way mentioned above.

Time mins.	Conversion N/10 NaOH cc.
20	1.75
40	2.93
60	3.75

Exp. 2. 1 g. taka-diasatase was dissolved in 5.0 cc. N/10 HCl (at 19°). After one hour it was neutralised with 5.0 cc. N/10 NaOH. Other conditions the same as in *Exp. 1.*

Time mins.	Conversion N/10 NaOH cc.
20	1.59
40	2.48
60	3.29

Exp. 3. Same as *Exp. 2*, only neutralised after two hours.

Time mins.	Conversion N/10 NaOH cc.
20	1.58
40	2.50
60	3.23

Exp. 4. 1 g. taka-diestase was dissolved in 5.0 cc. N/5 HCl + 5.0 cc. N/5 NaOH. Other conditions the same as in *Exp. 1.*

Time mins.	Conversion N/10 NaOH cc.
20	1.78
40	2.95
60	3.80

Hydrogen ion concentrations.
 I = 9.1×10^{-12} II = 8.5×10^{-9} III = 4.2×10^{-8} IV = 9.1×10^{-8} V = 4.0×10^{-7}
 VI = 1.1×10^{-6} VII = 8.5×10^{-6} VIII = 2.6×10^{-5} IX = 1.9×10^{-4}

CONDITIONS OF PROTEOCLASTIC ACTION OF TAKA-DIASTASE 135

Exp. 5. 1 g. taka-diastrase was dissolved in 5.0 cc. N/5 HCl. After one hour it was neutralised with 5.0 cc. N/5 NaOH. Other conditions the same as in *Exp. 1.*

Time mins.	Conversion N/10 NaOH cc.
20	0.73
40	1.33
60	1.60

Exp. 6. Same as *Exp. 5*, only neutralised after two hours.

Time mins.	Conversion N/10 NaOH cc.
20	0.70
40	1.22
60	1.55

Exp. 7. Same as *Exp. 5*, only neutralised after three hours.

Time mins.	Conversion N/10 NaOH cc.
20	0.65
40	1.10
60	1.45

From the above results one may judge that taka-diastrase is not very sensitive to acid. During contact with hydrochloric acid below N/10 = 0.365 %, it loses its activity but little, on contact with N/5 HCl the diminution of its action is considerable; but this degree of acidity never happens in clinical cases. Longer or shorter duration of contact with acids, so far as relates to differences of one to three hours, has not so much influence as difference in degree of acidity. Hence, taka-diastrase probably regains its action as soon as the medium resumes a suitable reaction. This justifies its clinical use.

As to the influence of salt on the proteoclastic function of taka-diastrase, we see from the above results that 1 g. taka-diastrase dissolved in 5 cc. N/10 HCl + 5 cc. N/10 NaOH or in 5 cc. N/5 HCl + 5 cc. N/5 NaOH has almost the same effect as that dissolved in 10 cc. distilled water, that is, taka-diastrase dissolved in N/10 NaCl solution or in water has the same efficiency. Therefore the influence of salt in such concentrations is negligible.

Finally, it seems necessary to determine whether the results obtained apply to the tryptic or ereptic action. Wohlgemuth states that taka-diastrase contains tryptic and ereptic ferments, like pancreatic juice; the action of the former is checked by blood serum while that of the other is accelerated

by it. Witte's peptone [Schjerning, 1898] contains, besides albumose, a certain amount of albumin; so that in the experiments given above, these two ferments may both have been in action. The following table seems to prove this fact.

TABLE II.

No.	Regulator mixture	P _H	Time mins.	Conversion N/10 NaOH cc.
1	2.0 cc. acetic acid ...	5.07	20	1.75
	2.0 cc. sodium acetate		40	2.85
	6.0 cc. distilled water		60	3.65
2	2.0 cc. acetic acid ...	5.12	20	1.70
	2.0 cc. sodium acetate		40	2.75
	1.0 cc. ox serum ...		60	3.46
	5.0 cc. distilled water		—	—
3	2.0 cc. acetic acid ...	5.10	20	1.40
	2.0 cc. sodium acetate		40	2.60
	2.0 cc. dog serum ...		60	3.05
	4.0 cc. distilled water		—	—

By adding blood serum to the regulator mixture a slight diminution of digestion was observed, though no important change in the reaction was produced. Since the diminution of the action is of a slight character, whereas that of trypsin itself should be strongly checked by serum, it seems reasonable to hold that the method used applies mainly to the ereptic function, the tryptic one being subsidiary.

CONCLUSIONS.

1. The optimal reaction for the proteoclastic ferments contained in taka-diastase is at $[H'] = 8.5 \times 10^{-6}$, i.e. they act better in a medium of slightly acid than in one of neutral or slightly alkaline nature.
2. These ferments are considerably resistant to acid; so that they regain their activity after neutralisation, if the acidity does not exceed the degree which usually obtains in the stomach of man or animals.

REFERENCES.

- Atkinson (1881), *Memoirs of Tokio Daigaku*. Quoted from Saito.
 Kellner, Mori and Nagaoka (1890), *Zeitsch. physiol. Chem.* 14, 297.
 Leo (1896), *Therap. Monatsch.* 10, 635.
 Michaelis and Davidsohn (1911), *Biochem. Zeitsch.* 36, 280.
 Saito (1906), *Centr. Bakt. Par.* II, 17, 20, 101, 152.
 Schjerning (1898), *Zeitsch. analyt. Chem.* 37, 413.
 Sørensen (1908), *Biochem. Zeitsch.* 7, 45.
 Strauss and Stargard (1898), *Therap. Monatsch.* 12, 65.
 Wohlgemuth (1912), *Biochem. Zeitsch.* 39, 324.