CV. DILATOMETRIC STUDIES IN ENZYME ACTION.

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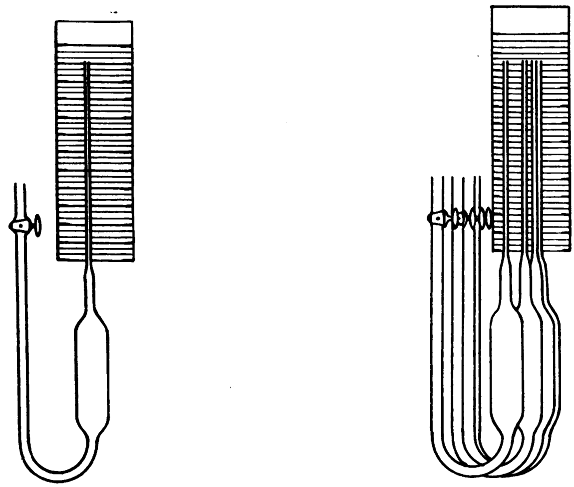
THE changes in volume accompanying enzyme actions are measurable and can therefore be studied with the aid of a dilatometer. Koelichen [1901] utilised the volume change to determine the velocity of decomposition of acetonyl alcohol in presence of sodium hydroxide. Benrath [1909] has made a study of the density changes of reacting liquid systems such as the inversion of sucrose and formation and hydrolysis of esters. Van't Hoff [1910] extended its application to a study of the enzymic synthesis of glucosides. Galeotti [1911] investigated dilatometrically the inversion of sucrose, the saponification of ethyl acetate, and the hydrolysis of starch, peptone and proteins by acids and in some cases by enzymes. Later [1912] he studied the conditions of synthesis of esters and fats brought about by pancreatic extract, employing the dilatometer. In spite of the lead given by the above authors the dilatometer does not appear to have been extensively employed in the investigation of enzymes, in spite of its simplicity and manipulative elegance and the accuracy of the results that can be attained.

THE DILATOMETER.

The instrument consists essentially of a bulb nearly 50 cc. in capacity. To one end is fused a tap, whilst the other end is connected to a capillary. The whole is bent into a U-form (see Fig. 1 (*a*)) the capillary-bearing bulb and the tap forming the two arms. Several such dilatometers can be used simultaneously by fixing them all by means of their capillaries to a suitable clamp (Fig. 1 (*b*)). A common scale, fixed behind the set of capillaries, allows the easy reading of the meniscus.

Agitation of the reaction mixture which is necessary in the case of insoluble enzymes like lipase, may be carried out by introducing a few glass beads into the bulb, before fusing on the tap, and mechanically shaking the dilatometer. A more elegant method is to introduce into the bulb a platinum-plated stirrer which can be worked electro-magnetically.



(a) (b)

Fig. 1.

EXPERIMENTAL.

The dilatometer is thoroughly washed successively with alcoholic potassium hydroxide, chromic acid, distilled water, alcohol and finally with ether, and then dried. The taps are carefully greased and a small reservoir of about 75 cc. capacity is attached to the tap end of the instrument by means of pressure tubing. By opening the tap and applying gentle suction at the capillary end, filling can be smoothly effected without the introduction of air bubbles.

The operation is carried out with the dilatometer and the reaction mixture completely immersed in the thermostat. Filling takes less than 5 minutes and the first reading can easily be recorded within that period. The temperature variation of the thermostat employed was $\pm 0{\cdot}001°$ and a control dilatometer which was always used served as an effective indicator of the constancy of the temperature maintained throughout the experiment.

The capacity of each of the dilatometers employed was determined and the capillaries were carefully calibrated in the usual way. A dilatometric study of the following enzyme reactions was carried out: taka-diastase on starch, invertase on sucrose, emulsin on salicin, tannase on methyl gallate and amidase on asparagine. In the case of diastase, the method has been extended to a study of the influence of salts and also to a determination of the rate of liquefaction. Every one of the dilatometrically investigated reactions has been simultaneously followed by an entirely independent method to establish the accuracy of the method.

Table I. *Hydrolysis of starch by taka-diastase.*

Materials: Lintner's soluble starch, 5 % solution; taka-diastase (Parke Davis), 1 % solution.
Mixture employed: 200 cc. starch solution + 5 cc. enzyme.
Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm. Control	Dilatometer readings, mm. Experimental	Maltose value cc. $KMnO_4$
30 mins.	108	122·5	4·50
45 „	108	118·5	5·90
65 „	108	111·5	7·30
95 „	108	105·0	9·35
132 „	108	96·5	11·65
220 „	108	80·5	15·80
295 „	108	69·5	18·20
364 „	108	61·5	25·55
24 hrs.	108	13·0	29·50
30 „	108	8·0	31·40

10 cc. of the reaction mixture were employed each time for Bertrand's estimation of maltose. Strength of $KMnO_4$: 1 cc. = 10 mg. Cu (see Fig. 2).

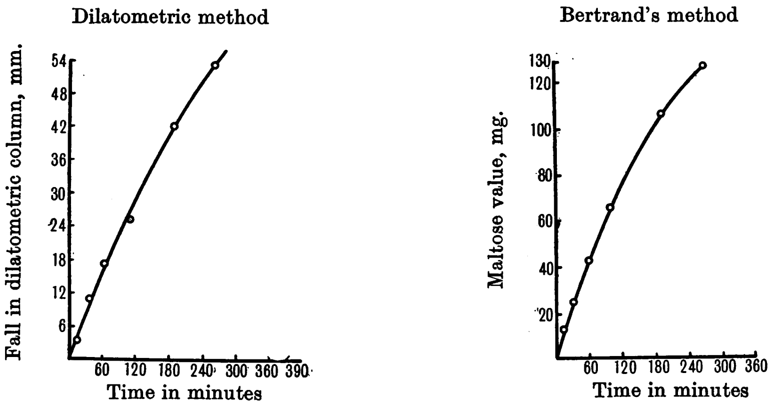


Fig. 2. Hydrolysis of starch by taka-diastase.

Table II. *Action of invertase on sucrose.*

Materials: sucrose solution, rotation + 5°; invertase, activity − 3·45°*.
Solutions employed: 300 cc. sucrose solution + 3 cc. enzyme; 300 cc. sucrose solution + 3 cc. boiled enzyme.
Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm. Control	Dilatometer readings, mm. Experimental	Polarimetric readings
9 mins.	385	232·5	4·50°
19 „	385	252·5	4·00°
28 „	385	283·0	3·51°
38 „	385	308·0	3·02°
44 „	385	324·0	—
49 „	385	339·0	1·93°
65 „	385	378·5	—
78 „	385	411·0	0·95°
91 „	385	441·0	—
104 „	385	469·0	0·51°
114 „	385	490·2	—
120 „	385	500·0	0·04°

* The fall in rotation brought about by unit volume (1 cc.) of the enzyme acting on 20 cc. of a 10 % solution of sucrose at 25° for 30 mins., measures the activity of the enzyme preparation [Sastri and Norris, 1928].

The two curves are superimposable (Fig. 3).

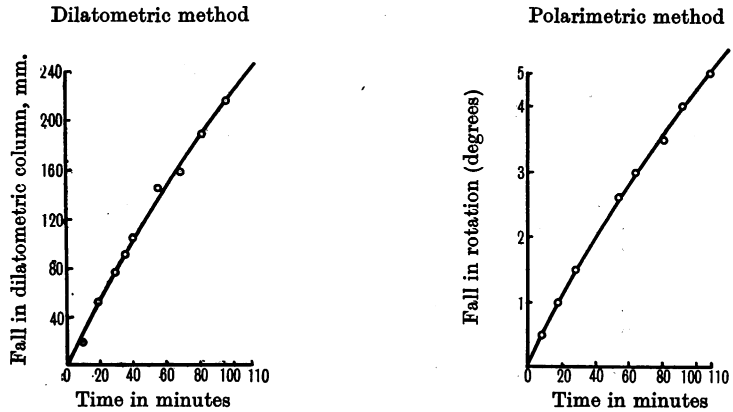


Fig. 3. Hydrolysis of sucrose by invertase.

Table III. *Action of emulsin on salicin.*

Materials: 1 % salicin solution (Kahlbaum's); 0·5 g. enzyme (B.D.H.) in 10 cc. water.
Solutions employed: experimental, 175 cc. salicin solution + 5 cc. enzyme; control, 175 cc. salicin solution + 5 cc. boiled enzyme.
Temperature of thermostat: 30·0°.

	Dilatometer readings, mm.		
Time	Control	Experimental	$KMnO_4$ cc.
22 mins.	214	406	—
34 ,,	214	392·5	—
75 ,,	214	363	14·55
94 ,,	214	354·5	—
120 ,,	214	347	17·20
155 ,,	214	340	—
157 ,,	214	—	18·50
207 ,,	214	334	—
213 ,,	214	—	19·70
267 ,,	214	330	—
273 ,,	214	—	20·00
332 ,,	214	325	—
387 ,,	214	—	20·10
392 ,,	214	324·5	—

The two curves are superimposable (Fig. 4).

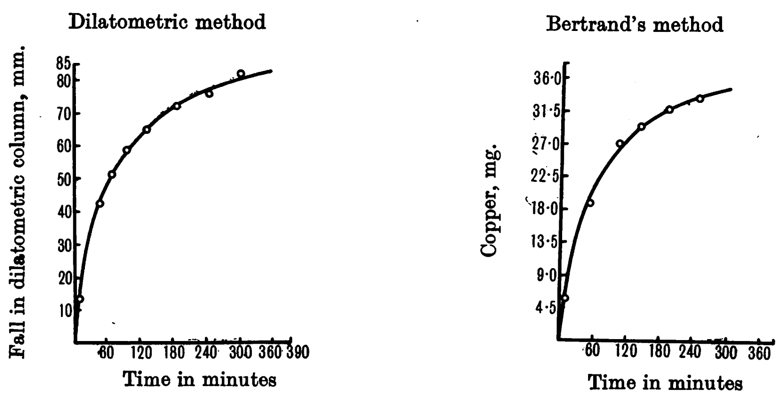


Fig. 4. Hydrolysis of salicin by emulsin.

Table IV. *Action of "cholam" amylase on starch paste.*

Materials: 1 % starch paste (Kahlbaum's potato starch); 1 % enzyme solution.
Solutions employed: experimental, 400 cc. starch paste + 16 cc. enzyme; control, 100 cc. starch paste + 4 cc. boiled enzyme.
Temperature of thermostat: 30·0°.

	Dilatometer readings, mm.		Viscometer readings.
Time	Control	Experimental	Time in secs.
12 mins.	206	285·0	—
32 ,,	206	285·5	—
45 ,,	206	—	118·0
47 ,,	206	287·0	—
72 ,,	206	288·5	—
75 ,,	206	—	116·0
105 ,,	206	292·1	—
110 ,,	206	—	114·5
185 ,,	206	—	110·5
260 ,,	206	299·0	—
265 ,,	206	—	107·5
320 ,,	206	299·5	106·2
440 ,,	206	303·0	105·4
720 ,,	206	307·5	105·0

(See Fig. 5.)

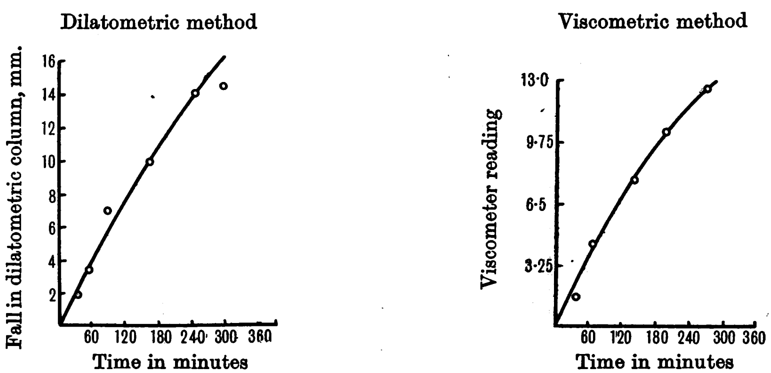


Fig. 5. Liquefaction of starch paste by "cholam" amylase.

Table V. *Hydrolysis of methyl gallate by tannase.*

Materials: 0·541 % solution of methyl gallate; enzyme extract, 10 g. mould in 150 cc. water.
Solution employed: experimental, 80 cc. methyl gallate solution + 20 cc. enzyme extract; control, 80 cc. methyl gallate solution + 20 cc. boiled extract.
Temperature of thermostat: 33·0°.

	Dilatometer readings, mm.		Electrometric titration
Time	Control	Experimental	cc. *N*/40 NaOH
0 hrs.	201	202·5	0·975
1 ,,	201	199·5	—
3 ,,	201	195·5	—
4 ,,	201	—	1·275
5·25 ,,	201	192·3	—
8 ,,	201	187·5	—
10 ,,	201	184·5	—
10·5 ,,	201	—	1·600
15 ,,	201	183·3	—
24 ,,	201	183·2	1·625
30 ,,	201	—	1·625

(See Fig. 6.)

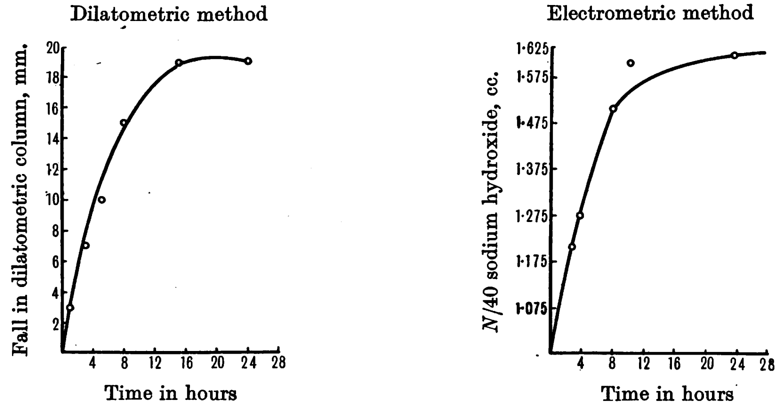


Fig. 6. Hydrolysis of methyl gallate by tannase.

Table VI. *Influence of salts on enzyme action.*

Solutions employed: experimental, 200 cc. of 2 % soluble starch, 10 cc. of 1 % taka-diastase, 10 cc. of $M/5$ potassium nitrate } in 250 cc.
control, without salt.

Time	Dilatometer readings, mm. Control	Experimental
22 mins.	415·0	405·0
37 ,,	407·5	391·0
52 ,,	402·0	384·0
75 ,,	396·0	378·5
105 ,,	391·5	374·0
145 ,,	388·5	370·0
185 ,,	387·5	—
210 ,,	—	367·5
260 ,,	384·0	—
265 ,,	—	366·0
340 ,,	—	365·0
385 ,,	381·5	—
19·5 hrs.	363·5	356·0

(See Fig. 7.)

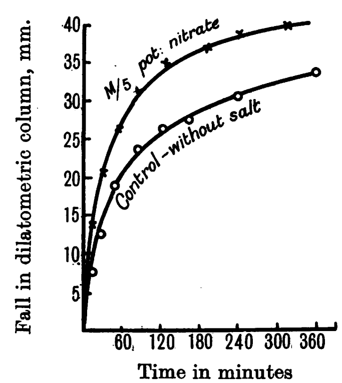


Fig. 7. Influence of potassium nitrate on the hydrolysis of starch by taka-diastase

Discussion.

A study of the tables and graphs demonstrates the closeness of the results obtained by the dilatometer and the methods generally adopted. In many cases, the dilatometric method has proved more satisfactory and less tedious. The estimation of sugars resulting during enzyme hydrolysis or the electrometric titration of acidities produced during the hydrolysis of methyl gallate and other esters is certainly more cumbersome and tedious than taking readings of the dilatometer at intervals.

The influence of salts like potassium nitrate on diastase has been studied. The study is being extended to other ions and colloids such as agar-agar, silicic acid, etc.

Limitations of the method. In cases where the volume change is not pronounced the method offers no special advantage over other methods. Again, the instrument cannot be employed in a study of enzyme reactions which involve the liberation of gases. In the course of an investigation of a preparation of amidase from *Aspergillus niger* on asparagine, no volume change was noticeable. The ammonia liberated during the enzymic cleavage of amides dissolves in the reaction mixture producing an increase in volume which is opposed to the fall due to hydrolysis. In such cases, accompanied by opposing changes of volume, this method is inapplicable.

Summary.

1. A convenient form of dilatometer for the study of enzyme action is described.

2. The hydrolytic action of diastase, invertase, emulsin, amidase and tannase on their respective substrates has been studied.

3. Limitations of the method are discussed.

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