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The Enzymic Activity of Egg-White. Its Bearing on the Problem of Watery-Whites

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One of the hypotheses advanced as an explanation of " watery-whiteness " in eggs is that there is present in the albumen of such eggs enzymes of proteolytic nature, by the action of which the protein becomes disintegrated, thereby decreasing in viscosity.

Experiments designed to test this hypothesis were commenced as far back as the spring of 1933, but the results then obtained were considered insufficient to substantiate the view, and the experiments were discontinued.

A publication appeared recently however by A. K. Balls and T. L. Swenson of the Bureau of Chemistry and Soils, Washington D.C. (1934), in which the claim was made that a proteolytic enzyme is present in egg-white, particularly in thick albumen, and that " the disappearance of thick white from eggs in storage is due to a slow proteolysis catalysed by tryptic proteinase ".

In view of the fact that the methods employed by these authors appeared in our opinion to be open to criticism, it was decided to investigate the problem using a suitable technique.

In the first place, a repetition of the Willstätter titration method employed by Balls and Swenson convinced us that the accuracy is insufficient for the detection of such small changes as were to be expected. In no instance could increases of the order observed by the American workers be recorded; the end-points were never particularly sharp in such large volumes. Attempts were made to increase the accuracy by titrating to a definite pH standard in a comparator instead of " to a faint but true blue which required a little practice to recognise " but without much success.

The choice of the above method for the purpose of the investigation would seem moreover to have been singularly unfortunate. Recognising, as it does, any increase in carboxyl groups, it would fail to distinguish between lipolytic and proteolytic activity. The presence of an active lipase in both egg yolk and egg white has been demonstrated by Koga (1923) among others.

Any attempt to detect the presence of small quantities of proteolytic enzymes (proteases, ereptases, etc.), in egg albumen demands the employment of a sensitive micro-method which is specific for the determination of peptide cleavage. Of the various methods available, the well-known formol titration technique appeared to us to be the most suitable. Using decinormal carbonate-free sodium hydroxide, contained in an automatic micro-burette graduated in twentieths of a cubic centimeter, and titrating to a standard pH value (pH 8·4) with the help of a Cole-Onslow comparator, no difficulty was experienced in attaining an accuracy of $\pm 0{\cdot}02$ c.c. This is clearly evident from the protocols recorded.

Experimental.

Having in our previous experiments (not recorded) employed only the one substrate gelatin, which is frequently used in the determination of tryptic activity, and obtained negative results, it was decided to extend the scope of the investigation by including such substrates as casein and peptone and by working over a wide pH range, any erepsin-like ferment would be expected to attack casein and peptone but not gelatin. No search was made for lipolytic or other types of enzyme.

Albumen solutions.

Various numbers of eggs were used as recorded, either new laid (1 hour old), fresh or old from white Leghorn pullets, intensively housed and all fed on a grain and mash ration. The albumen was separated into the two portions, thick and thin by the sieve method of Holst and Almquist (1931). 3 c.c. portions of either fraction were diluted to 10 c.c. with water and allowed to stand for 30 minutes at 35°.

In the majority of these experiments, the precipitated mucin was centrifuged off after shaking the diluted solution with glass beads, thereby avoiding the difficulty of removing a representative sample of the digestion mixture by the pipette, and excluding the possibility of precipitated particles adsorbing the indicator.

It is noteworthy that the proportion of thick to thin albumen did not appear to be markedly different in normal and so-called watery-white eggs, except those which were very stale, leading us to suspect that physical variations in organisation of the protein phase may be intimately bound up with the phenomenon of watery-whiteness.

Substrates.

Two and 5 per cent. casein solutions were prepared in the usual way by grinding the protein with the requisite amount of alkali, and diluting to volume. The pH was adjusted if necessary to 8·4.

Two per cent. gelatin was prepared by dissolving commercial gelatin in warm water (60°) and adjusting the pH.

2 per cent. peptone was similarly prepared from Parke, Davis & Co., " Bacterologic Peptone ".

A few drops of toluol were added and each stoppered solution kept in the ice box when not in use

Buffers.

A range of phosphate and of borate buffers were prepared from Clark and Lubs tables; the solutions were not, however, diluted as a high buffering power was desired. In the comparator a borate buffer of pH 8·4 was employed.

Trypsin.—An active trypsin solution was prepared from pigs pancreas according to Cole (1926). Its activity was tested upon casein (see control experiment No. 4).

A glycerol extract of pancreas was also prepared and this trypsinogen utilised to check the activity of the enterokinase preparation (calcified milk method).

Enterokinase was prepared following Balls and Swenson (1934).

An Erepsin solution was prepared by grinding pig's intestinal mucosa with sand and water, straining and filtering. Its activity is demonstrated in control experiments Nos. 6 and 7.

Formol Solution.—This was made up according to Cole (1926). One drop of 1 per cent. alcoholic phenolphthalein solution being added per c.c. and sufficient sodium hydroxide solution to bring to pH 8·4. The reaction was carefully adjusted each day.

Titration Technique.—The digestion mixtures were invariably made up by mixing, in small stoppered flasks, 10 c.c. of the diluted egg albumen, 10 c.c. of substrate solution and 15 c.c. of buffer and adding 4 drops of toluol, an amount previously determined as sufficient to inhibit bacterial activity.

In cases where kinase was used, 1 c.c. of this solution replaced 1 c.c. of buffer. The buffering power was sufficiently strong to enable this to be done without affecting the final pH of the mixtures. The solutions were left in a constant temperature water bath at ±37°.

An aliquot of 10 c.c. was withdrawn for titration at zero time. To this was added 10 drops of 1 per cent. alcoholic phenolphthalein solution and decinormal sodium hydroxide run in until the colour as seen in the comparator matched the standard of pH 8·4. Five c.c. of formol solution was now added and the mixture again titrated to pH 8·4, the quantity required being a direct measure of the amount of amino nitrogen present. The delicacy of the titration was such that a difference of one drop, equal to 0·02 c.c., could be easily distinguished.

After a time interval which varied in different experiments from 1 to 24 hours, a second sample was removed and similarly titrated, a final sample being taken at the close of the experiment. The titration differences between the zero and first and second time intervals are recorded in the protocols.

Results.

Control Experiments.

The control experiments of Table I served to demonstrate the reliability of the method as far as reproductivity of results was concerned and the absence of bacterial interference. Even in the

presence of enterokinase no proteolysis occurred in the various substrates after 24 to 46 hours, which justified the conclusion that the increases in amino nitrogen observed when egg albumen or enzyme solutions were present could be attributed to the action of the latter.

Erepsin was active upon casein and upon peptone but had no effect upon gelatin.

The use of casein as a substrate by Balls and Swenson can be criticised on the grounds that it fails to distinguish between erepsin-like enzymes and true proteases (e.g. trypsin). The conclusion arrived at by these authors that a " tryptic proteinase " is present in eggs is not justified by their data, if one discounts the reported auxiliary effect of entero-kinase. In some cases no increase occurred on the addition of kinase, in others, slightly higher titration figures were recorded, but considering the limitations of the method these figures do not, to our mind, carry much conviction.

Autolysis Experiments.

Were a protease responsible for the breakdown of egg albumen, leading to the condition of watery-white, it would be expected that measurements of amino nitrogen in such albumen solutions, suitably buffered, would reveal the presence of such an enzyme especially when incubated at a temperature of 37° for periods ranging from 6 to 24 hours.

It seemed natural to look, in the first place, for such autodigestion of egg albumen alone. Autolytic activity of tissues usually proceeds most rapidly in a slightly acid medium. Tests were however performed with thick albumen solutions buffered at reactions ranging from pH 5·5–6·0; 7·5–8·0. No action was observed at any pH although these eggs were shown to contain an active erepsin as subsequently reported (see Table II, trial 12).

Autolytic proteinases seem, therefore, to be definitely absent from the eggs under consideration in our experiments.

In order to meet the possibility that a proteinase was present but that egg albumen was not a suitable substrate to reveal its activity, experiments were carried out in which egg albumen was added to gelatin solutions buffered at pH 7·8 or 8·4 and with, or without, the addition of kinase. No increase of amino nitrogen could be demonstrated (see trials 1, 9 and 13). The egg concerned in trial 1 was a " watery-write " as understood by the trade.

That the majority of eggs do contain an erepsin-like enzyme particularly associated with the thick albumen was clearly proved by the use of casein or peptone solutions as substrates. The activity is not great and requires a micro-method for its demonstration. The enzyme would appear to be associated in normal eggs with the thick albumen fraction; even in a five-months-old egg a feeble action was found in the thick white but none in the thin. In eggs having floating air cells, this enzyme was demonstrable in both fractions (see trials 6 and 8). In the case of trial 6, the albumens from two eggs were mixed and a greater activity found in the thin white than

the thick. It must be remarked, however, that in one of these eggs only a small quantity of thick white failed to pass the sieve. It is possible that a diffusion of the enzyme had occurred from thick to thin.

It is significant that proteolysis was observed upon peptone and casein as substrates without the addition of enterokinase.

As regards the pH optimum, the preliminary trials Nos. 2-11 appeared to indicate a greater activity at pH 7·8 than at 8·4. It was decided, therefore, to observe the activity of individual albumen solutions, pooled from two eggs, over a sufficiently wide pH range.

These results are recorded in trials 14-16 and Figs. 1, 2, and 3; Fig. 4 representing a composite of the final changes observed in the individual experiments.

It seems clear that two regions of maximum activity exist; the one fairly sharply localised at pH 5·5, the other spreading over the range pH 7·0-8·0. At intermediate reactions a certain amount of activity could still be detected.

It seems to us more likely that two separate enzymes having different optima are concerned, and this suggestion is borne out by the greater relative activity in the pH 7·0-8·0 region in the case represented by Fig. 1, no such difference occurring in the other two cases. It is well known that a mixture of two enzymes having different optima will afford an extended pH-activity curve exhibiting two humps. As an example, the pH-activity curve of pepsin acting upon egg albumen (Northrop 1919) has been selected and reproduced twice upon the same graph paper so that the two curves lie symmetrically about the point of their intersection. This gives a hypothetical case comparable with the conditions obtaining in egg white. It is seen from Fig. 5 that the overlapping results in a composite curve, exhibiting two maxima and an extended intervening range of lesser activity. We consider, therefore, that the curves of Fig. 4 may each be resolved into two partially overlapping components, representing two enzymes whose optima lie at approximately pH 5·5 and 7·0-8·0.

Discussion.

Several records are to be found in the literature of investigations upon the enzymic activity of eggs, but in many cases the results were not unambiguous.

Wohlgemuth (1905) reported the presence of a proteolytic enzyme in egg-yolk, but was unable to demonstrate any such activity in egg albumen. Koga (1923) denies the presence of a trypsin-like enzyme in yolk, detecting, on the other hand, a not-inconsiderable ereptic activity. Egg white, he considers, contains a fibrinolytic enzyme in small amount. Additional references are quoted by Balls and Swenson.

It is evident from the present work that at least two peptide-splitting enzymes were present in the albumens of the eggs studied, whether normal or " watery-white ". The enzyme with a pH optimum about 7·6-8·2 is probably similar to tissue erepsin.

Although our data do not pretend to offer any explanation of the cause of " watery-white " it is of interest to discuss our findings in the light of some of the hypotheses which have been put forward to explain this condition.

In the first place the conclusions of Balls and Swenson are clearly untenable. It is difficult to understand, however, how an ereptic enzyme could in any way modify the condition of the native protein. Our autolysis trials indicate that no breakdown occurs under these conditions at pH values ranging from 5 to 8.

It is also significant that a number of eggs examined within 12 hours of laying have been found by us to exhibit the condition known commercially as " watery-whiteness ". Storage is not essential to the development of this condition and in our opinion the condition induced by shaking, as reported by various authors (e.g. Canham 1934, Halnan 1933, Dryden 1934), is not identical with that which appears in certain eggs a few hours after having been laid.

Our observations suggested some association between a tendency towards natural watery-whiteness and imperfect shell formation. In support of this, it may be recorded that imperfectly shelled eggs picked at random from a day's collection proved subsequently, although not in every case, to develop watery-whiteness within a short time after being laid (12 hours), and our experience would indicate that experiments taking cognisance of this fact, might help to throw light upon the problem.

Few, if any, eggs with perfectly developed shells of fine texture and good calcification, result ultimately in watery-whites.

The white of an egg is an elaborately organised structure as may be verified by observing its behaviour when poured upon a sieve. The thick albumen forms a kind of pouch supported no doubt by interlacing fibrillæ of mucin. Within this pouch the bulk of the thin white is contained and is liberated when the structure gives way upon the sieve surface. Even so, both thick and thin fractions contain mucin which becomes visible upon diluting the materials with water.

As stated previously, a low proportion of thick to thin albumen is by no means an invariable accompaniment to the watery-white condition, in fact our experiments have shown that this is rarely the case. The lowered viscosity of the white seems to be due largely to a loss of structural organization. It would be of interest to determine whether the total amount of mucin in watery-white eggs is less than in normal eggs and also what alterations, if any, this protein has undergone.

Artificial disintegration of the albumen, as for example by the injection of trypsin or erepsin into normal eggs, may be expected to result in a lowered viscosity of the white, but this in no way proves that the naturally occurring watery white is produced in the same way.

A hypothesis which makes some appeal to us is that the cause may be sought in an upset of the bound- to the free-water ratio. Proteins are known to combine with a certain proportion of the water in which they are dissolved in such a manner that this quantity of the solvent is bound or eliminated in such a way that it no longer participates in osmotic phenomena. Any release of this bound water causes a diminution in the viscosity of the system. The point is susceptible of experimental investigation and should be considered in any attempt to explain the occurrence of " watery-whites ". St. John (1931) has found the bound water in thick egg white to be no less than 26 per cent.

Regarding the whole problem from a detached standpoint, it appears to us that many tacit assumptions have been made which require experimental proof or disproof. Speculation based on false premises is obviously useless. Physico-chemical possibilities have not received the attention they would appear to merit in such a problem. It seems to us that the first point to be elucidated in an approach to the problem is the actual nature of the difference between the colloidal systems of protein in water comprising the total white in normal and watery-white eggs. The function of the mucin appears to be to preserve a structural organization in the white as between the albumen and the other members of the colloidal complex, but how this function is discharged is obscure. Some disorganization seems to take place in " watery-whiteness ".

One cannot help recalling the somewhat similar problem of gluten dispersions in water. The protein from a " strong " flour forms a highly viscous colloidal solution, that from a " weak " flour a less viscous one. The reason for this behaviour is not known. Even a highly viscous solution undergoes a more or less rapid spontaneous decrease in viscosity without, as far as can be ascertained, any marked or obvious change in the protein; the action of enzymes has been definitely excluded.* Our knowledge of protein systems with their possibilities of hydration, dissociation and polymerisation is still so meagre that much work will have to be done before such peculiar changes as that above mentioned can be understood. It is along these lines, however, that we feel the solution of the watery-white problem will ultimately be attained.

Summary.

1. The enzymic activity of solutions of thick and thin albumen from normal and watery-white eggs has been studied, using a micro-titration method determining peptide cleavage in terms of the increase in amino nitrogen.

2. Thick or thin albumen alone at pH values ranging from 5·5 to 8·5 suffer no autolysis at 37°, neither can any protein-splitting enzyme be detected at any pH by the addition of gelatin as substrate.

* Since this was written, an article has appeared by Blagoveschenski and Yurgenson (1935) in which it is claimed that there is present in wheat flour an enzyme which exercises a definite solvent action on these proteins by virtue of a disaggregation rather than an hydrolysis. It is claimed that there is no increase in amino-nitrogen during the process. It would seem highly desirable that egg white be studied in a similar manner.

3. The work of Balls and Swenson is criticised on account of insufficient sensitivity of the method employed and the fact that it is such as would fail to distinguish between an increase in acid groups due to lipolytic and due to proteolytic activity. Moreover, the use of casein as a substrate is incapable of differentiating protease from ereptase activity.

4. The presence in egg white of at least two erepsin-like enzymes having different pH optima (approximately 5·5 and 7·0-8·0) has been demonstrated.

Whether or not these enzymes play any part in the development of " watery-white " is uncertain but for reasons discussed it would seem unlikely

Some observations upon " watery-white " in eggs are recorded and the problem subjected to a short discussion.

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TABLE I.

Substrate.	Enzyme.	Corresponding Trial No.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
					Hours.		
Gelatin 2%....	—	1	7·8	-	18	Nil	No action.
					42	Nil	
Casien 5%.....	—	7	7·8		2·5	Nil	No action.
					24	Nil	
Peptone 2%...	—	3	7·8		1	Nil	
					46	Nil	No action induced by added kinase.
				+	1	Nil	
					46	+0·02	
Casein 5%.....	Trypsin		8·4	—	1	0·07	0·2 c.c. extract of fresh pancreas.
					46	0·72	
	Erepsin		8·4		0·5	Nil	No clotting of calcified milk in 18 hours.
					18	Nil	1 c.c. extract in intest. mucosa.
Casein 2%.....	Erepsin		7·8		0·5	+0·15	Definite action on casein at 7·8.
					18	0·90	
Peptone 2%....	Erepsin		8·4	+	0·5	0·20	Definite action on peptone at pH 8·4.
					18	1·05	
Gelatin 2%....	Erepsin		8·4		0·5	Nil	No action.
					18	+0·05	

TABLE II.

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
								Hours.		
1	Gelatin 2%	1	2 days	Thick	47·7	8·4		1	−0·35	This egg had a floating air-cell
								5	−0·35	
								1	−0·02	Albumen not centrifuged.
								5	−0·03	Negative results, probably due to bad sampling. 1 c.c. kinase used.
								1	Nil	
				Thin				5	Nil	
								1	+0·03	No action.
								5	+0·02	
2	Casein	3	1 hour	Thick	44·6	8·4	—	1	−0·03	
								5	Nil	Membranes centrifuged out. 1 c.c. kinase used.
							+	1	−0·02	
								5	−0·02	
								1	−0·02	No action.
								5	−0·02	
				Thin			+	1	+0·02	
								5	+0·02	
3	Peptone 2%	Same three eggs as in experiment No. 2		Thick				2	+0·10	Performed day following Experiment No. 2. Albumen solution kept in refrigerator. 2 c.c. kinase used. An ereptase probably present; action increased by kinase. Greater action with thick white.
								21·5	+0·30	
								2	+0·30	
								21·5	+0·43	
								2	+0·02	
				Thin				21·5	+0·17	
								2	+0·05	
								21·5	+0·19	
4	Peptone 2%	3	1 day	Thick	48·0	7·8		1	+0·13	An ereptase probably present. 1 c.c. kinase had no auxiliary effect.
								21	+0·25	
								1	+0·12	
								21	+0·22	
								1	Nil	No action with *thin* white.
								21	−0·02	
				Thin				1	Nil	
								21	+0·04	

TABLE II. (*continued*).

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
								Hours.		
								1·5	+0·10	
				Thick				18	+0·15	Feeble action with *thick* white only.
								1·5	+0·05	
5	Casein 5%	3	5 months		41·9	8·4		18	Nil	
								1·5	—	1 c.c. of kinase used which had no auxiliary effect.
								18	−0·15	
				Thin				1·5	—	
								18	−0·25	
								18	+0·10	These eggs had floating air-cells. In one, practically no thick white left. 1 c.c. kinase used. Action greater in the *thin* white than in the thick.
				Thick				66	+0·25	
							+	18	+0·10	
6	Casein 5%	2	4 days		41·9	7·8		66	+0·15	
								18	+0·30	
								66	+0·40	
				Thin			+	18	+0·18	Kinase had no auxiliary effect.
								66	+0·36	
	Casein 5%					7·8		2·5	+0·25	No action on peptone at pH 8·4. Definite action on casein at pH 7·8. 2 c.c. kinase used.
7		1	1 hour	Thick	43·4			24	+0·20	
	Peptone 2%					8·4		2·5	+0·07	
								24	−0·10	
	Casein 5%					7·8	+	2·5	+0·30	This egg had a floating air-cell. Conclusions of former trial confirmed.
8		1	1 day	Thick	45·2			24	+0·25	
	Peptone 2%					8·4		2·5	+0·05	
								24	−0·10	2 c.c. kinase used.
	Gelatin 2%					7·8		2	+0·02	No action on gelatin or casein at either pH.
								22	−0·04	
						8·4		2	−0·09	Definite action on peptone at pH 7·8.
								22	Nil	
9	Casein 2%	3	1 hour	Thick	53·8	7·8		2	−0·15	2 c.c. of kinase used.
								22	Nil	
						8·4		2	−0·07	
								22	+0·05	
	Peptone 2%					7·8		2	0·03	
								22	+0·25	

TABLE II. *(continued).*

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
								Hours.		
						7·8		2	Nil	Definite action on both substrates but more marked at the lower pH. Similar results on peptone at pH 7·8 when mucin not removed. 2 c.c. kinase used.
							+	22	+0·37	
10	Casein 2%	2	11 days	Thick	45·0	8·4		2	Nil	
								22	+0·05	
	Peptone 2%					7·8		2	+0·15	
							+	22	+0·33	
						8·4		2	+0·11	
								22	+0·23	
						7·8		1·5	+0·15	Definite action on both substrates in each case; activity appears to be less at the lower pH values. 2 c.c. kinase used.
								20	+0·46	
11	Casein 2%	2	1 hour	Thick	52·7	6·5	+	1·5	+0·15	
								20	+0·35	
	Peptone 2%					5·0		1·5	Nil	
							+	20	+0·03	
								6	−0·01	No action at any pH although these eggs known to contain an active enzyme: see previous experiment.
						5·5	−	24	−0·02	
								6	−0·01	
		Same eggs as in experiment No. 11				6·0	−	24	−0·01	
								6	+0·02	
12	Nil			Thick		6·5	−	24	+0·02	There is no evidence of an autolytic protease acting on the albumen as substrate.
								6	+0·03	
						7·0	−	24	+0·03	
								6	+0·03	
						7·5	−	24	Nil	
								6	+0·04	
						8·0	−	24	Nil	
	Gelatin 2%					7·8		24	Nil	No action on gelatin. Definite action on peptone. No kinase used.
								98	Nil	
13	Peptone 2%	2	1 hour	Thick	50·0	6·0		20	+0·08	
								44	+0·11	

TABLE II. (*continued*).

Trial No.	Substrate.	Number of eggs.	Age.	Kind.	% thick white.	pH.	Kinase.	Time.	Increase c.c. N/10 NaOH.	Remarks.
								Hours.		
						5·0		20	Nil	Definite action on peptone; apparently two optima at pH 5·5 and within the range pH 7·0–8·0. (See Fig. 1).
								48	Nil	
						5·5		20	+0·17	
								48	+0·29	
						6·0		20	+0·07	
								48	+0·23	*For pH 8·5 borate buffer used and the figures corrected to the same basis as phosphate buffer.
						6·5		20	+0·11	
								48	+0·42	
14	Peptone 2%	2	1 hour	Thick	45·8	7·0		20	+0·07	
								48	+0·47	
						7·5		20	+0·13	No kinase used.
								48	+0·67	
						8·0		20	+0·11	
								48	+0·58	
						8·5 (Borate)		20	+0·13*	
								48	+0·22	
						5·0		24	+0·09	Definite action for first 24 hours, little progress after this time. Two optima at pH 5·5 and range pH 7·0–8·0. (See Fig. 2.)
								48	+0·11	
						5·5		24	+0·17	
								48	+0·17	
						6·0		24	+0·10	
								48	+0·10	All reagents sterilized by autoclaving and the amount of toluol present increased.
15	Peptone 2%	2	1 hour	Thick	53·8	6·5	—	24	+0·11	
								48	+0·11	
						7·0		24	+0·10	No kinase used.
								48	+0·23	
						7·5		24	+0·15	
								48	+0·10	
						8·0		24	+0·20	
								48	+0·22	
						5·0		24	+0·02	Definite action on peptone. Two optima at pH 5.5 and range pH 7·0–8·0.
								48	+0·05	
						5·5		24	+0·23	
								48	+0·31	(See Fig. 3.)
						6·0		24	+0·19	
								48	+0·27	No kinase used.
16	Peptone 2%	2	1 hour	Thick	50·0	6·5	—	24	+0·13	
								48	+0·25	
						7·0		24	+0·23	
								48	+0·23	
						7·5		24	+0·10	
								48	+0·20	
						8·0		24	+0·12	
								48	+0·28	

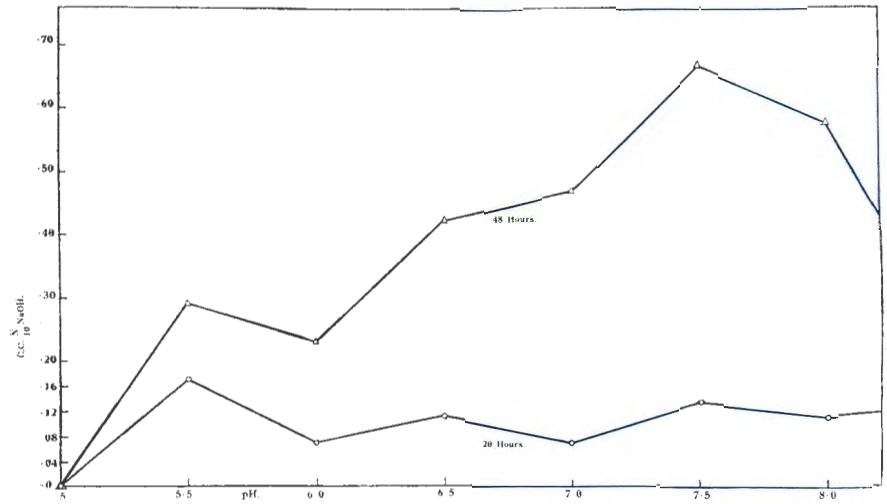
·70
·60
·50
·40
·30
·20
·16
·12
·08
·04
·0
c.c. N/10 NaOH.
48 Hours.
20 Hours.
5
5·5
pH.
6·0
6·5
7·0
7·5
8·0

Fig 1.

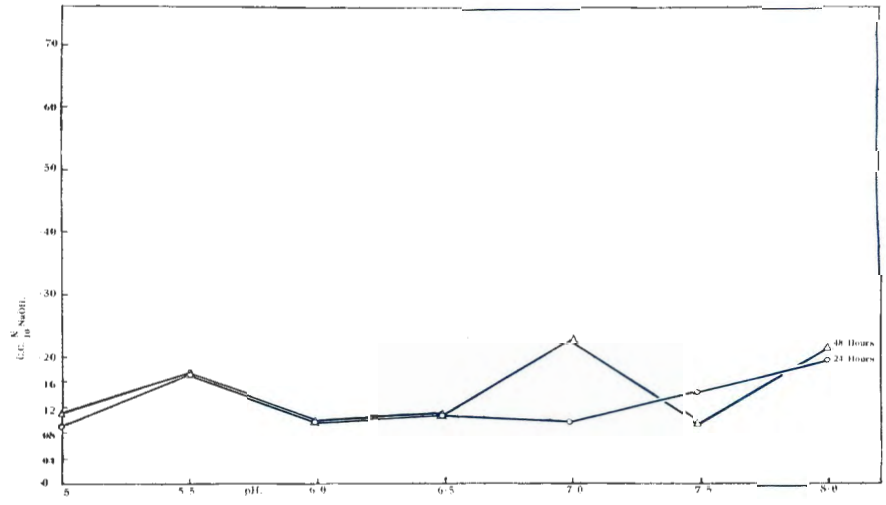
70
60
50
40
30
20
16
12
08
04
0
c.c. N/10 NaOH.
48 Hours
24 Hours
5
5·5
pH.
6·0
6·5
7·0
7·5
8·0

Fig. 2.

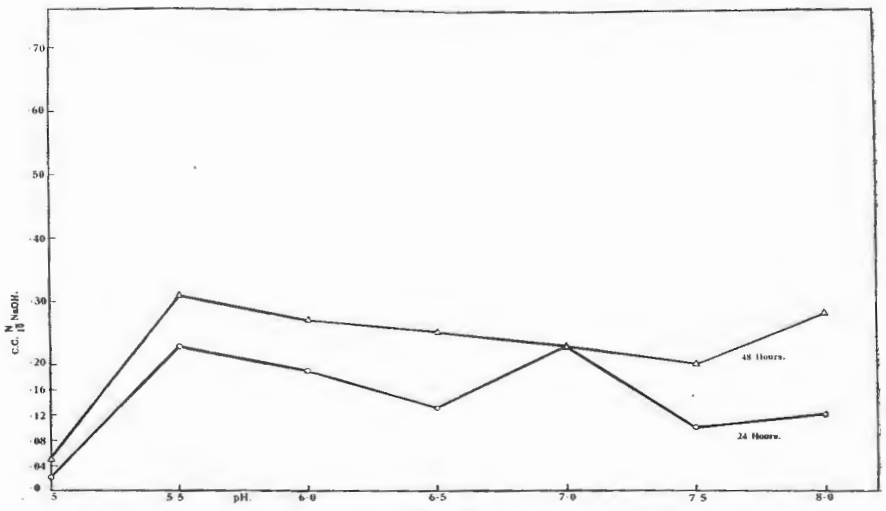
·70
·60
·50
·40
·30
·20
·16
·12
·08
·04
·0
c.c. N/10 NaOH.
48 Hours.
24 Hours.
5
5·5
pH.
6·0
6·5
7·0
7·5
8·0

Fig. 3.

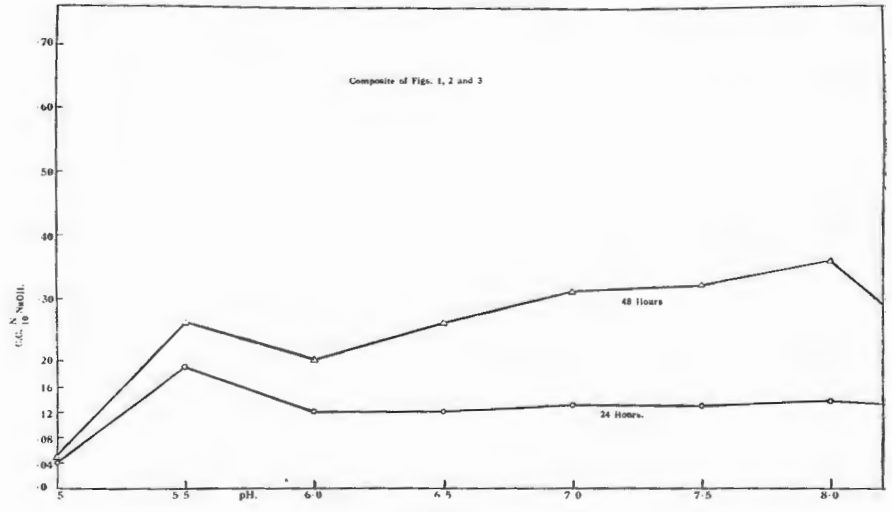
Composite of Figs. 1, 2 and 3
·70
·60
·50
·40
·30
·20
·16
·12
·08
·04
·0
48 Hours
24 Hours.
5
5·5
pH.
6·0
6·5
7·0
7·5
8·0

Fig. 4.

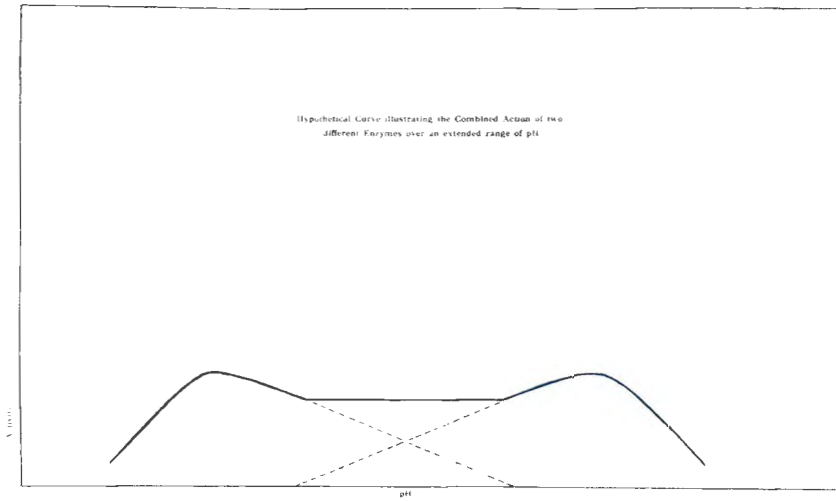
Hypothetical Curve illustrating the Combined Action of two
different Enzymes over an extended range of pH
pH

Fig. 5.