It remains to consider the distinct effect produced by galactose.

On reference to the diagrams figs. 6 and 7 representing the spatial arrangement of the atoms in the two sugars, it will be noticed that the only difference between them is that the hydrogen atom X shown to the left of the oxygen atom in the ring is in a plane behind the ring in the one case, and in a plane in front of the ring in the other; there is also a corresponding difference in the relationship of the linking oxygen atom to the ring plane. The difference between the close packed assemblages, therefore, would probably be small: though sufficient perhaps to reduce the compatibility of the two molecules, some degree of compatibility might still persist.

This is one of those cases of minute difference which it will be important to study further, especially in view of the observation made by more than one worker that some yeasts "acquire" the power of fermenting galactose if habituated to its presence. The question of the presence of a distinct enzyme in emulsin capable of hydrolysing milk sugar and presumably of inducing the synthesis of β-galactosides must also be reconsidered from this point of view: we are at present engaged in this inquiry.

Studies on Enzyme Action. XXI.—*Lipase* (III).

By H. E. Armstrong, F.R.S., and H. W. Gosney, B.Sc.

(Received June 13,—Read June 26, 1913.)

On account of the part which Lipase plays in promoting the resolution of fats generally into fatty acid and glycerol, one of the most important processes in animal nutrition, it is desirable that a clear picture should be obtained of the manner in which the activity of the enzyme is exercised.

The material hydrolysed—the fat—being practically insoluble and the enzyme presumably a colloid, the interaction to be considered is that of substances insoluble in water and therefore presents unusual features.

Two brief communications on the subject were made to the Society in 1905 and 1906.* In the first of these, which had reference to the enzyme in castor oil seeds, it was stated that Connstein's contention had been confirmed that the presence of acid is necessary to condition the hydrolysis of a fatty oil by the enzyme and that practically any acid was effective provided a sufficient amount were used. As acids did not act equally in

* 'Roy. Soc. Proc.,' B, vol. 76, p. 606; vol. 78, p. 376.

equivalent quantities, although when used in sufficient amount the weak were as effective as the strong, it appeared to be probable that the strength of the acid was a factor in the action.

The enzyme could not be separated from the seed. An attempt that was made to liberate it by treating the residue left after extracting the seed with sulphuric acid and removing the excess of acid by washing thoroughly was unsuccessful: apparently the enzyme was destroyed.

The final conclusion arrived at was that the *Ricinus* enzyme is possessed of properties which make it specially capable of promoting the hydrolysis of glycerides of the higher fatty acids.

In the second communication, results were quoted which again were an indication that the strength of the acid used in promoting the hydrolysis of fats was a factor of importance and that, in the case of the stronger acids used, no action took place when more than a certain proportion of acid was present.

The conclusion of chief importance arrived at in this communication was that comparable results could only be obtained, in the case of ethereal salts soluble in water, by using solutions of equivalent concentration: from which it follows that no comparison can well be made between soluble and insoluble ethereal salts.

When soluble salts were compared, it was found that the enzyme was the more active the less soluble the ethereal salt and the weaker the acid from which the salt was derived. Thus ethylic succinate was hydrolysed to a considerable extent and the far more soluble allied ethylic tartrate (dihydroxysuccinate) was scarcely if at all affected,* the salt of intermediate solubility, ethylic malate (monhydroxysuccinate), being acted upon less readily than the succinate but more readily than the tartrate.

In explanation of these results, it was suggested that the attachment of the enzyme to the carboxylic centre of the ethereal salt—the necessary first act in the process of hydrolysis—was *interfered with by the hydration of this centre,* the implication being that hydration took place the more readily and to a greater extent the more soluble the ethereal salt.

Various results were quoted in this communication showing that, whilst it was less active towards fats than *Ricinus* lipase, liver lipase determined the hydrolysis of various ethereal salts without any addition of acid: though attention was not directed specially to this point, on this account and because of the retardation of hydrolysis by acids when used in excess, the opinion had

* We are inclined to think that the slight amount of action observed in this case is due to the fact that the acid liberated by the direct action of water on the tartrate prevents hydrolysis by the enzyme.

been formed that the acid served merely to liberate the enzyme from *Ricinus* seed and was not directly effective as a co-enzyme in promoting the hydrolysis of the ethereal salt. In other words, it appeared to be probable that the zymogen in the seed was merely a salt which became active when "neutralised" by an acid.

Since 1906, at intervals, many attempts have been made by one of us to arrive at an understanding of the peculiarities of Lipase but it has been possible only recently to interpret the results in a simple and consistent manner, so as to correlate the effects produced by this enzyme with those observed in the case of other natural hydrolysts: in fact until the views which are put forward in the previous communication had taken definite shape and a clear conception had been formed of the manner in which enzymes generally effect hydrolysis, it was difficult, if not impossible, to formulate an explanation of the manner in which an enzyme operates when acting under such peculiar conditions and to appreciate the relative significance of the various observations made with Lipase.

Preparation of the Enzyme.—The most important advance made in recent years in connexion with Lipase is the discovery by Yoshio Tanaka* that an active enzyme may be prepared by digesting pressed castor oil seed with a proper amount of acid, then washing to remove all soluble matter. This material is most active in a neutral medium, less so in the presence of acid, especially mineral acid.

In a more recent communication, published in September 1912,† it is stated that the optimum quantity of acid is 5 c.c. of N/10 sulphuric or 6–7 c.c. of N/10 acetic acid for each gramme of the pressed seed. The method recommended is to digest 100 grm. of pressed or extracted castor oil seed with 600–700 c.c. of the acetic or 500 c.c. of the sulphuric acid at 30–35° and after about 30 minutes to wash the residue thoroughly with water and then dry the pasty mass at a temperature not exceeding 40°. If oil be digested at 30–35° with 3–4 per cent. of the dried Lipase powder thus prepared and 6–10 times as much water as powder, about 90 per cent. of the glyceride is hydrolysed within 7–10 hours.

In our experience, the enzyme prepared with a very weak acid such as acetic is distinctly superior to that obtained when a stronger acid is used: the enzyme appears to be in some way altered by "fixation" of the acid and the effect cannot be counteracted by neutralisation with alkali.

We have usually prepared the enzyme by crushing the shelled castor oil

* 'Journ. of the College of Engineering, Tokyo Imperial University,' 1910, vol. 5, No. 2, p. 25.

† *Ibid.*, No. 4, p. 125.

seed in a mortar and digesting the oily mass with petroleum spirit: after 24 hours, the greater part of the oil is removed by squeezing it through calico cloth. The residue is treated twice in the same manner, using ordinary ether instead of petroleum spirit; it is then ground up in a mortar and digested during about 15 minutes with 80 c.c. of N/10 acetic acid to every 10 grm. of the meal. The liquid having been filtered off, the residue is washed several times by alternately transferring it to a beaker containing water and pouring off the liquid through a filter: it is then dried in a vacuum desiccator and, when dry, ground up and sifted through fine muslin. Succinic acid can be substituted for acetic but when tartaric acid is used the product is less active. During the washing process about 40 per cent. of the material goes into solution. The amount of powder obtained is about 9 per cent. of the weight of the seeds. Our preparation has not proved to be so active as that described by Tanaka but we have obtained better results with it than with a preparation made according to his directions.

Experimental Method.—The hydrolytic experiments were all carried out in 50 c.c. Jena hard glass flasks closed with rubber stoppers. These were maintained at a constant temperature in a Hearson incubator, 14 by 12 by 12 inches, heated electrically and provided with an electrically controlled regulator.

In order to agitate the contents of the flasks during the experiments, they were fixed by rubber bands to paddles carried on a central steel shaft, $\frac{3}{8}$ inch in diameter, passing through the horizontal axis of the chamber; this shaft was rotated at a rate of about six revolutions per minute by means of a small motor outside the chamber. The shaft carries a square metal block attached to which are four brass fins at right angles to each other. A wooden plate, $4\frac{3}{4}$ by 10 inches, about $\frac{1}{4}$ inch thick, forming the paddle, is screwed to each of the brass fins; a narrow strip of wood is fixed near the edge of the paddle and grooves are cut in this to receive the necks of the flasks; the bodies of the flasks rest in corresponding holes cut in the paddles. The arrangement will be obvious from the figure on p. 590.

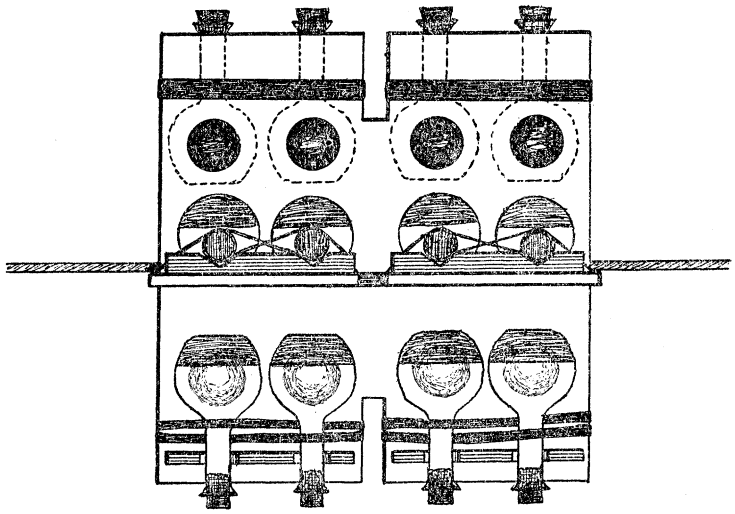
The titrations were carried out in 50 per cent. alcoholic solutions, using normal or N/10 alkali and phenolphthalein.

Acidity of the Enzyme.—The untreated oil-free seed residue is distinctly acid, neutralising from 2 to 4 c.c. of N/10 alkali per gramme; whilst it hydrolyses succinic ether nearly as well as the acid treated material, it has very little action on fatty oils.

When either acetic or succinic acid is used and the meal is washed until the filtrate is no longer acid to phenolphthalein, the product is still acid, that prepared with the aid of acetic acid requiring from 4 to 5 c.c. of N/10 alkali

per gramme to neutralise it; when tartaric acid has been used, as much as 7–8 c.c. of alkali may be required; the product obtained with the aid of sulphuric acid has a still higher acidity and is also less active.

Very little if any acetic acid is absorbed during the treatment but the amount of tartaric acid fixed is considerable: thus in one experiment, using



5 grm. of the residue, on titrating the washings, it was found that 15 out of 40 c.c. of N/10 acid were retained: on using N/10 sulphuric acid, 22·5 out of 40 c.c. were absorbed.

Effect of Acid on the Activity of the Enzyme.—The following results were obtained on contrasting the action on 10 c.c. of M/20 solution of succinic ether, to which different amounts of glycine had been added, of 0·5 grm. of enzyme prepared with the aid of tartaric and acetic acid respectively: in the latter case, two different preparations were tested.

Molecular proportions of glycine	Percentage hydrolysed by enzyme prepared with tartaric acid	Percentage hydrolysed by enzyme prepared with acetic acid	
		a	*b*
0	42·2	58·5	50·8
½	42·7	57·6	51·4
1	46·5	61·3	53·3
2	48·5	63·5	56·9
3	—	67·0	
4	58·5	—	62·6
5	—	70·2	
6	64·4	—	68·0

These results show that the recovery of activity, even in presence of a considerable excess of the "basic" material, is only moderate; they appear to justify Tanaka's conclusion that the enzyme is more active under neutral than under acid conditions.

The inferiority of the tartaric product is equally obvious when tested by means of oil. Thus on digesting 0·5 grm. of the powder with 5 grm. of olive oil and 2 c.c. of water during 20 hours, the amounts hydrolysed were as follows:—

	Enzyme *a* prepared with acetic acid	Enzyme prepared with tartaric acid
(1)	77·6 per cent.	45·1 per cent.
(2)	75·5 „	48·4 „

To test the effect of concentration, three 5 grm. samples of oil-free meal were digested with equal volumes (40 c.c.) of solutions of tartaric acid of different strength (N/5, N/10, N/40) and after washing and drying, 0·5 grm. of each product was digested with 10 c.c. of a solution of succinic ether. The amount of hydrolysis effected in 20 hours was:—

Untreated enzyme	51·1 per cent.
Digested with N/5 acid	30·9 „
N/10 „	41·5 „
N/40 „	8·5 „

As it was possible that insufficient acid had been used in the case of the N/40 solution, a constant amount of tartaric acid but different volumes of the solution were used.

Enzyme prepared with the aid of	20 c.c. N/5 acid	hydrolysed	42·6 per cent.
	40 „ N/10	„	43·9 „
	80 „ N/20	„	54·4 „
	160 „ N/40	„	46·1 „

The following two tables afford further illustrations of the influence of acids:—

Table showing the Effect of increasing Amounts of various Acids on 0·5 grm. of treated Enzyme acting during 20 hours on 10 c.c. M/20 Solution of Succinic Ether.

Concentration of acid	Glycine	Butyric	Succinic	Malic	Tartaric	Sulphuric
No acid	58·5	56·5	60·3	58·7	60·7	55·7
N/40	57·6	60·5	56·7	60·3	58·8	8·1
N/20	61·3	61·8	55·8	—	52·6	
N/10	63·5	57·3	55·0	56·6	33·3	
N/5	70·2	44·2	55·6	51·1	5·2	
N/3·3	—	17·0	53·6	21·2	—	

Table showing the Effect of the Presence of Four Equivalents of various Acids on the Action of 0·5 grm. of treated Enzyme on 10 c.c. M/20 Solution of Succinic Ether, *i.e.*, in N/5 Acid Solutions.

	Percentage hydrolysed
Glycine	71·0
Acetic acid	63·4
Succinic acid	62·0
No acid	62·0
Butyric acid	50·9
Malic acid	41·3
Citric acid	29·6
Tartaric acid	15·8
Sulphuric acid	0·8

To hydrolyse succinic ether, a relatively large amount of the enzyme is required. Thus on digesting 1 grm. of the Lipase powder with 10 c.c. of an M/20 solution of ethylic succinate (0·087 grm.) during 48 hours, the ethereal salt was completely hydrolysed: but ½ grm. of the powder was insufficient.

The enzyme is only slightly, if at all, affected by the action and will hydrolyse fresh ester but its action on oil is somewhat impaired. Thus when 0·5 grm. of enzyme was allowed to act during 20 hours on four different quantities, each 10 c.c. M/20 ethylic succinate, in succession, hydrolysed

52·0	per cent.	during	1st use
50·5	,,	,,	2nd ,,
48·4	,,	,,	3rd ,,
47·6	,,	,,	4th ,,

The once used enzyme, however, caused only 29·5 per cent. hydrolysis of 5 grm. of oil, the fresh enzyme causing about 66 per cent. in the same time. This lipoclastic weakening of the enzyme is possibly due to a loss of emulsifying power.

Whatever the amount of enzyme present, the amount of action appears to be approximately proportional to the amount of ethereal salt in solution. Thus 0·5 grm. enzyme acting during 20 hours at 30° C. hydrolysed:—

0·0096	grm.	ethereal	salt	in 10	c.c.	M/100 solution
0·0234	,,	,,	10	,,	M/50	,,
0·0327	,,	,,	10	,,	M/33·3	,,
0·0487	,,	,,	10	,,	M/20	,,
0·0710	,,	,,	10	,,	M/15	,,
0·0913	,,	,,	10	,,	M/10	,,

But apparently, when undissolved ethereal salt is present, the amount of action is not greatly in excess of that effected in saturated solution. Thus, on digesting 5 grm. of succinic ether with 5 c.c. of water and 0·5 grm. of enzyme during 20 hours, only 0·205 grm. was hydrolysed—or about twice as much as when an M/10 solution was used.

When the amounts of ethereal salt and enzyme were kept constant the amount hydrolysed decreased as the solution was diluted.

Thus in 5 c.c. M/10 solution	+	0 c.c. water	62·5	per cent. was hydrolysed
2	„	59·4	„	„
5	„	61·2	„	„
10	„	53·5	„	„
20	„	40·1	„	„
40	„	15·4	„	„

Thus everything tends to show that lipase is very sensitive to the action of acids, though acids are produced by its action. Its inferiority as a hydrolyst of ethereal salts other than fats and its power of hydrolysing the complex natural glycerides readily, apparently to an almost unlimited extent, would seem to be due to the fact that the acids that are liberated from fats are scarcely if at all soluble in water and very weak.

Mode of Action of Lipase.—The argument previously put forward (Part II) in explanation of the activity exercised by lipase is as follows:—

"The ethereal salts which are hydrolysed under the influence of lipase are all compounds of the type R′.CO.OX′. Since R′ and X′ may be varied within wide limits, it cannot well be supposed that the selective action of the enzyme is exercised with reference either to R′ or X′; consequently, the controlling influence must be attributed to the carboxyl radicle (CO.O): the enzyme must be so constituted that it can 'fit itself to this group.'

"Our experiments have led us to form the provisional hypothesis that the hydrolysis of the ethereal salt by Lipase involves the direct association of the enzyme with the *carboxyl centre,* and that such association may be prevented by the 'hydration' of this centre: consequently, that those salts which are the more attractive of water will be the less readily hydrolysed."

The behaviour of soluble ethereal salts, especially the fact they are the less readily acted upon the more soluble they are and the stronger the acid from which they are derived, may be better accounted for, however, by assuming *not* that the carboxylic centre of the ethereal salt is hydrated and that therefore the association of the enzyme with this centre is *prevented:* but that salt and enzyme are the more kept apart, through the agency of water, the more soluble the salt is, because the salt is the more attracted by the water; and that the enzyme is the more interfered with, through the fixation of acid, the stronger the acid.

It remains to account for the special affinity of lipase to fats. Owing to the fact that it acts on carboxylic ethereal salts in general, it cannot be supposed that it has any special attractive influence over the molecule of the hydrolyte as a whole, such as is pictured in the previous communication as

exercised by the enzymes which hydrolyse glucosides, for example, though the fact that it acts preferentially on fats cannot be left out of account. It would seem to be necessary to assume only that the enzyme is one in which an attractive carboxylic centre is freely exposed.

To judge from the properties of acids generally, it would seem that the carboxylic group has a marked tendency to combine with itself—thus acetic acid appears to exist under ordinary conditions as a polymerised form of the fundamental molecule $CH_3.CO.OH$; and, judging from their slight solubility, this is true also of a great many acids. The *free* carboxyl radicle, CO.OH, probably has marked attractive power and far greater activity than is apparent in acetic acid, for example.

It is a question, therefore, whether the properties of lipase may not be accounted for on the assumption that it is a colloid molecule possessed of a carboxylic or even a phosphoric group so situated that it cannot be self-neutralised but yet sufficiently near to a basic centre to be interfered with by any acid which can combine with this latter.

It may well be that the configuration of the enzyme is such as specially to favour its association with glycerides of the higher fatty acids. But the association of enzyme and hydrolyte is doubtless determined by an intervening water film, *i.e.* the carboxylic centres in the two compounds are both to be thought of as hydrolated and as brought into contact through the agency of the attached water molecules. The number of molecules thus activated will depend on the osmotic conditions which prevail in the mixture undergoing change.

We are under the impression that the lipase powder contains an emulsifying constituent and that its activity is perhaps in no small measure dependent on this constituent. It is impossible to say at present whether the intrinsic acidity of the powder prepared by means of a weak acid which is not retained to any appreciable extent is that of the enzyme proper or of a practically insoluble acid associated with it: we are inclined to think that the intrinsic acidity of the powder is to be correlated with its emulsifying power. As the decrease in the activity of the enzyme when it has a high acid value is equally marked towards succinic ether, however, the inferiority cannot well be attributed solely to loss of emulsifying power.

Influence of the Products of Change.—Under ordinary conditions the hydrolysis of fats by lipase powder is incomplete—partly perhaps because the action is reversible but mainly, we think, on account of the retarding influence of the products of change and the decay of the enzyme.

With regard to the influence of the acid liberated from a fat on the course of change, the conclusion arrived at recently by Tanaka will be obvious from the following quotation :—

"Different amounts of soya bean oil were hydrolysed by the 'lipase powder' in the presence of different amounts of fatty acid at 38° C. The results were as follows:

Oil	Acid	Enzyme	Water	Grammes of oil hydrolysed		Percentage of oil hydrolysed	
				1 hour	2 hours	1 hour	2 hours
grm.	grm.	grm.	grm.				
50	0	2	18	22·00	32·50	44·0	65·0
40	10	2	18	17·73	26·33	44·1	65·5
30	20	2	18	13·13	20·31	43·2	66·8
10	40	2	18	4·70	7·33	43·5	67·9

"These data show that the amount of oil hydrolysed is directly proportional to the amount of oil present or the rate of change follows the law of mass action, even in the presence of a large amount of free fatty acid. This result proves without doubt that the fatty acid has no retarding effect upon the enzyme action, because, if that were the case, it would lead to a slowing of the reaction greater than that due to the diminished concentration of the neutral oil."

Tanaka's experiments do not appear to us to justify these conclusions. It will be noticed that the four interacting substances were all present in different *relative* proportions in each of the four experiments—consequently the results are not comparable.

It is not to be expected that an oil will behave as an aqueous solution, but there must be some optimum proportion of enzyme to oil beyond which the oil will be in excess, as it is obvious that any influence the products of change can exercise will be relatively greater the smaller the proportion of oil present.

That the extent to which hydrolysis takes place is not proportional to the amount of oil used is shown by the following results of a series of experiments in which 1 grm. of enzyme and 4 c.c. of water were digested during 20 hours with varying amounts of oil:—

Oil used	No. of c.c. N/NaOH neutralised	Per cent. of oil hydrolysed
10 grm.	18·6	54·5
20 „	26·3	38·1
30 „	28·9	28·0
50 „	35·6	20·6

In a second experiment in which 25 c.c. of water was used together with 1 grm. of enzyme, the results were as follows:—

10 grm.	21·1	61·8
20 „	29·6	43·3
30 „	33·8	32·8
50 „	39·8	23·2

It will be noticed that in both cases only about twice as much oil was hydrolysed when the amount used relatively to a given weight of enzyme was increased five times.

The amount of oil hydrolysed is also not proportional to the amount of enzyme used: thus on digesting 5 grm. of oil and 30 c.c. of water at 30° C. during three days the percentage hydrolysed was:—

By 0·5 grm. enzyme	58·5
„ 1·0 „ „	83·0
„ 1·5 „ „	92·0

Apparently, when sufficient water is present, an increase in the amount produces relatively little effect. Thus on digesting 10 grm. of oil with 1 grm. of enzyme and varying amounts of water during 20 hours, the results obtained were as follows:—

Water used	Percentage hydrolysed
3 c.c.	88·2
5 „	90·3
10 „	90·5
25 „	90·1
50 „	89·7

Oleic acid is strong enough to make the untreated enzyme active if used in sufficient amount. Thus on digesting 5 grm. of oil, 2 c.c. of water and 0·5 grm. of oil-free seed with varying amounts of oleic acid, the results obtained were:—

Molecular proportion of oleic acid	Percentage hydrolysed
0	1·6
0·5	3·2
1	5·8
2	23·4
4	32·8
6	35·7

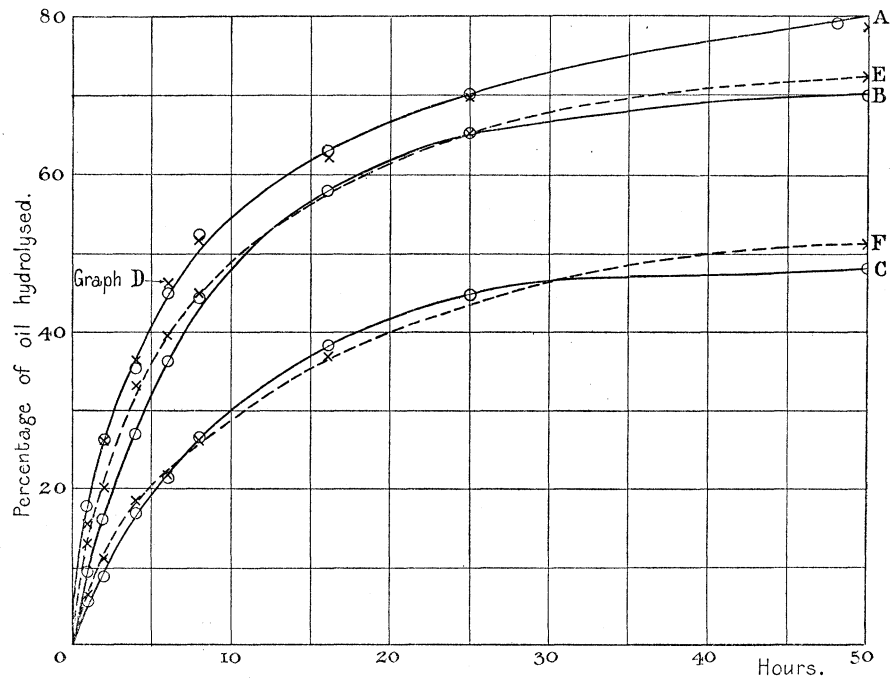
But oleic acid has a marked retarding effect. Thus in 20 hours the percentage of oil hydrolysed in presence of different proportions of the acid by the enzyme prepared with the aid of acetic acid was as follows:—

0	80·6
0·5	82·4
1	84·3
2	70·9
4	58·1
6	45·6

The retarding effect of oleic acid is shown more clearly in the following table and diagram representing the results obtained in experiments in which the rate of change was contrasted in presence of various proportions of one or other or both products of change :—

Table showing Percentage of Change effected by 0·5 grm. of Enzyme acting on 5 grm. of Olive Oil and 2 c.c. Water at 30° C. (O = 1 molecular proportion of Oleic Acid; G do. Glycerol.)

Time in hours	Oil alone Graph A	Oil + 3 O Graph B	Oil + 6 O Graph C	Oil + G Graph D	Oil + 2 G Graph E	Oil + 3 O + G Graph F
1	17·9	9·5	5·6	15·7	13·0	6·6
2	26·3	16·1	8·9	26·1	20·1	11·3
4	35·4	27·0	16·9	36·4	33·1	18·5
6	44·9	36·4	21·2	46·1	39·6	21·8
8	52·3	44·2	26·3	51·6	44·8	26·1
16	62·8	57·9	38·2	61·9	57·2	36·8
25	70·1	65·2	44·5	69·8	65·2	44·5
50	78·9 (48 hours)	69·7	47·7	78·3	72·2	51·1



We are inclined to attribute the slight effect glycerol produces to alterations in the osmotic conditions affecting the enzyme-oil interface. On the

other hand, we regard the powerful retarding effect of oleic acid as a case of the preferential retardation referred to in the previous communication, this being promoted by the solubility of the acid in the oil; apparently, it is too weak an acid to interfere as such.

As it is conceivable that the reduction of the rate of hydrolysis in presence of the acid may be due in part at least to the effect it has in promoting the reversal of the interaction, we have endeavoured to ascertain if the change can be carried to completion by removing the glycerol as it is formed. To this end, the hydrolysis was carried out in a cell constructed by stretching parchment paper over the opening at the narrow end of a small bell-jar. An emulsion of 50 grm. of oil, 20 c.c. of water and 5 grm. of enzyme was placed within the jar, which was surrounded with water; the oily mixture was constantly stirred by means of a glass stirrer actuated by a fan kept in rotation by means of a small flame.

After about 24 hours, a curd or clot separated from the mixture and only 67·7 per cent. of the oil was hydrolysed after three days. Apparently the enzyme had been either destroyed or in some way modified and rendered inactive. This result was confirmed in a second experiment in which the mixture was kept at rest.

Observers differ as to the effect of use on lipase. It appears to be easily changed if left in contact with water and this probably is the reason why different preparations differ more or less considerably in activity. The following results may be quoted in this connexion.

1·5 grm. of lipase powder was shaken up with water and the mixture left at 30° C. during two days in a diffusion thimble immersed in water. As a control, 1·5 grm. of the powder was merely digested with the same volume of water in a flask. Toluene was added in each case. The water in the beaker remained clear during 24 hours but was cloudy after 48 hours. The solid was then filtered off and its action tested as follows.

A fourth part of each enzyme preparation was added to 5 grm. of oil and portions of the water used for dialysis, etc., as given in the following table, water being added to make up the volume to 18 c.c. in each case:—

	Percentage hydrolysed in 24 hours
1. Untreated acid prepared enzyme	36·3
2. Enzyme from diffusion thimble	6·1
3. Ditto + liquid from outside of cell	4·6
4. Ditto + liquid from inside cell	9·1
5. Enzyme merely digested with water	8·3
6. Ditto + water with which it had been washed	8·8

But the activity of the enzyme seems also to deteriorate during its action on oil, as the following results show:—

		Oil hydrolysed Grammes
5 grm. of oil + 2 c.c. of water + 0·5 grm. of lipase powder digested during 5 days at 30° C.	*a.*	3·50
	b.	3·49
As in *a* and *b* but after 48 hours' digestion 5 grm. of oil + 2 c.c. of water were added and the digestion extended to 5 days	*c.*	3·66
	d.	3·88

Evidently the enzyme had been to a large extent destroyed during the first period of digestion in experiments *c* and *d*.

In view of the possibility that the enzyme does not deteriorate when used to hydrolyse succinic ether in the way that it does when used with a fatty material might be due to the fact that the acid liberated, in the former case, is much stronger than the higher fatty acid and therefore serves to preserve the lipase from destruction by inhibiting the action of proteoclastic enzymes, a second similar series of experiments was made, using 10 c.c. of water in the one set and 10 c.c. of N/10 succinic acid in the other. Practically the same results were obtained with oil and water alone as before but only about one-third as much oil was hydrolysed in presence of the acid and there was no increase after adding the second portion of oil.

What has been said in this section will serve to show that the difficulties attending the study of the action of lipase on fats are considerable. We may here point out that we are inclined to attribute the observed inferiority of liver lipase as a hydrolyst of fats to the unnatural conditions under which it is applied when the material used is the expressed juice from an animal organ. Under natural conditions the lipase and fat are in close conjunction, not suspended in water.

When the graphs given in the diagram on p. 597 are inspected, it is obvious that at first the action proceeds at a rapid rate. Therefore, taking into account the influence of the products of change and the fact that the enzyme diminishes in activity during use, it is not improbable that far from being in accordance with the law of mass action, the change proper proceeds in the manner which appears to be characteristic of other enzymes—*i.e.* the graphs representing the rate of change would be nearly linear, if the disturbing influences could be allowed for.

As to the nature of the enzyme, it is difficult to formulate any definite conception but we are inclined to extend the argument advanced in the previous communication and to take the view that, as already suggested, the configuration of lipase is such as to favour its association with glycerides of the higher fatty acids—in other words, that it contains a glyceric nucleus

attached to a carboxylic centre in proximity to an acidic group which can determine the hydrolysis of a fatty molecule which becomes attached to the glyceric nucleus. It is conceivable that a single carboxylic centre in conjunction with the glyceric nucleus would be sufficient to constitute an *acceptor* of fats; this would allow of the attachment of the glyceric nucleus to a colloid complex and leave the third carbon atom free to combine in some other way: if a phosphoric residue became associated with this carbon atom, it would probably serve as *agent*. Such an assumption is in accordance with the instability of the enzyme, it may be added.

Summary.

Following directions given by Yoshio Tanaka, a method is described of rendering the lipoclastic enzyme in castor oil seed active by treatment with dilute acid—preferably acetic acid.

It is suggested that the "zymogen" is merely a salt.

Much evidence is quoted showing that the activity of the acid treated enzyme is interfered with even by dilute acids and that it is easily rendered inert by excess of acid.

It is contended that lipase is specially fitted to hydrolyse the oily glycerides of the higher fatty acids and is not suited to act in aqueous solutions. The interaction must be supposed to take place at and between surfaces separated only by a thin film of water at most.

It is shown that the products of change, both the fatty acid and the glycerol, especially the former, inhibit the interaction of enzyme and oil.

As the rate at which interaction takes place is dependent presumably on the conditions at the colloid surface and these cannot be expressed in terms of the concentration of the solution, it is impossible to apply the law of mass action to the interpretation of the changes observed. Probably, as in other cases of enzymic action, a given amount of enzyme changes equal amounts of material in successive equal intervals of time, the observed departure from this rate being due to the inhibiting effects of the products of change and also to the destruction of the enzyme.