IX. THE STABILITY OF PANCREATIC LIPASE.

By DAVID HENRIQUES DE SOUZA.

From the Physiological Laboratory, King's College, London.

(Received February 3rd, 1916.)

For some years glycerol extracts of pancreas have been extensively used in this laboratory by Rosenheim, Shaw-Mackenzie and others. The lipase contained in the extracts has been the centre of interest, but the presence of other enzymes and the bearing this may have on the behaviour of lipase have not been overlooked. The most important of these enzymes, in this respect, is trypsin, which has often been shown to be present in these glycerol extracts.

Conflicting statements with regard to the action of trypsin on lipase appear in the literature. Experiments with pancreatic juice carried out by Terroine [1910] and by Mellanby and Woolley [1914] show that lipase rapidly disappears in presence of trypsin. Mellanby and Woolley state further that glycerol extracts of pancreas contain trypsinogen and enterokinase, and that activation is inhibited by the glycerol; but that dilution removes this inhibitory influence and trypsin is formed which destroys the lipase present in the extract. Shaw-Mackenzie [1915], on the other hand, claims that trypsin is present in the concentrated extract, and Tsuji [1915] has shown that dilution not only does not abolish, but may even increase, the lipoclastic activity.

These contradictory statements leave us in a state of uncertainty as to the stability of the lipase in glycerol extracts of pancreas. All the extracts used in this laboratory have been strongly lipoclastic and those tested have been found to be proteoclastic. An extract has been employed for class work for the last two years and has suffered no deterioration in its fat-splitting power during that time. Dr Shaw-Mackenzie has kindly supplied me with

notes showing that some of the extracts prepared by Rosenheim and himself were active more than two years after they were made. As several of these extracts have been kept, I have taken the opportunity to investigate further the stability of the lipase.

EXPERIMENTAL.

Preparation of the extracts.

All the extracts were prepared in the same way. Fresh pig's pancreas was freed from fat, minced and weighed. It was then allowed to stand for 24 hours with glycerol, 2 cc. of glycerol being used for 1 g. of pancreas. The extract was strained off through two layers of muslin, and kept in a stoppered bottle. Just before use one part of this concentrated extract was diluted with two parts of water.

Estimation of lipoclastic activity.

The method employed was that used by Rosenheim and Shaw-Mackenzie [1910]. 5 cc. of an olive oil emulsion were incubated with 3 cc. of the dilute extract at 37° for 18–24 hours and the fatty acids estimated by titration with N/10 NaOH, alcohol being added to facilitate titration and phenolphthalein being the indicator. The estimations were done in duplicate and the means of the results were corrected for the original acidity of the mixture of emulsion and extract. The details of the method have recently been very fully described by Umeda [1915]. I find, with him, that the quality of the oil is of importance. The length of time during which the mixture of extract and emulsion is shaken has also to be considered. Bradley [1909] has investigated the effect of shaking emulsions, and his results show that, if an emulsion be shaken too long, lipoclasia is lessened. He suggests in explanation that aëration destroys the enzyme or makes it difficult for the enzyme to reach and act upon the oil. While this may be the explanation in many cases, some of my experiments show that, in shaking the mixture of extract and emulsion, a difference in time of only ten seconds causes the oil to separate out, thus producing a condition which should retard lipoclasia. In the following experiment it will be seen that shaking for ten seconds gave a much better result than shaking for twenty seconds.

Exp. 1. Flasks containing 3 cc. of dilute extract and 5 cc. of oil emulsion were shaken for 5, 10, and 20 seconds, respectively, and put into the incubator at 37° for 22 hours. The mixture, before incubation, required 2 cc. of

N/10 NaOH to neutralise it. This number was subtracted from those obtained after incubation, and the corrected numbers are given.

Time of shaking, secs.	N/10 NaOH cc.
5	32.8
10	35.2
20	20.4

The oil had separated out in the last flask only. With an oil resistant to lipase this difference is not appreciable as the numbers obtained are all small. This may be due to the fact, pointed out by Umeda [1915], that the oil contains an impurity which resists the action of lipase even when the oil is finely emulsified. Such oils, too, often form thin emulsions, and when mixed with the extract they separate out in the incubator, or, more rarely, on being shaken for a short time. The inappreciable differences produced by shaking, and the low numbers obtained with these oils, are evident on comparing the following with the preceding experiment, the extract used being the same in both cases.

Exp. 2. Pairs of flasks containing 3 cc. of dilute extract and 5 cc. of oil emulsion were shaken for 5, 10, and 20 seconds, respectively, and put into the incubator at 37° for 18 hours. The mixture, before incubation, required 1.1 cc. N/10 NaOH to neutralise it. The mean of the titration numbers for each pair, after incubation, was taken, and these means were corrected for the original acidity. The final numbers are given.

Time of shaking, secs.	N/10 NaOH cc.
5	3.3
10	3.4
20	3.1

In order that differences due to shaking might not vitiate the experiments, the mixture of extract and emulsion was shaken slowly ten times in all the subsequent work.

Estimation of tryptic activity.

The tryptic activity was measured by Sørensen's [1908] formaldehyde method, decinormal sodium hydrate being used for titration.

The following was the usual procedure:

3 cc. of dilute pancreatic extract and 10 cc. of a 2 % solution of caseinogen in 0.4 % sodium carbonate were put into each of three flasks. The amino-acids were at once estimated in one of these by the addition of 10 cc. of neutral formalin and subsequent titration with N/10 NaOH. The other two

flasks, after the addition of 0.5 cc. of toluene to each, were put into the incubator at 37° for 18–24 hours. The amino-acids were then estimated in the same way. The mean of the numbers obtained for these two was taken, and from it was subtracted the number obtained for the control flask. The result was a measure of the proteoclastic activity.

The activity of old extracts.

Ten extracts which had been prepared at periods varying from nearly six to three years ago were examined, and their lipoclastic and proteoclastic powers compared with those of an extract three months old (No. 11 in the series) and known to be active. The extracts were diluted before use for the lipoclastic but not for the tryptic determinations, and the experiments were carried out in the manner already described, lipoclasia going on at 37° for 18 hours, and proteoclasia for 24 hours. Each experiment was performed in duplicate and the mean of the results corrected for the original acidity of the mixture of extract and substrate. For the numbers illustrating the lipoclastic activity (after 18 hours at 37°) of the extracts soon after preparation I am indebted to Dr Shaw-Mackenzie. The proteoclastic activity at that time was not recorded. The numbers are collected together in the following table.

No.	Lipoclastic activity in cc. N/10 NaOH		Time interval between (I) and (II)		Tryptic activity in cc. N/10 NaOH
	(I)	(II)	yrs.	mths.	
1	14.8	31.2	5	8	6.3
2	23.6	26.6	5	7	4.9
3	28.9	20.7	5	4	3.9
4	16.8	15.9	5	4	3.9
5	12.6	5.2	5	4	2.6
6	14.1	24.1	5	3	3.7
7	28.1	26.5	5	2	5.4
8	13.3	26.6	4	10	4.3
9	39.2	15.5	3	11	3.8
10	25.8	27.6	3	4	5.4
11	—	30.0	—	—	7.4

(I) shows the original and (II) the present lipoclastic activity. The numbers in (I) are not strictly comparable with each other as the estimations were not all made with the same oil; those in (II) are comparable with each other, having all been obtained under the same conditions, but are not strictly comparable with those in (I). It is evident, however, that all the extracts are still lipoclastic and, except No. 5, compare favourably with the recent preparation No. 11. No. 5 is a thin extract and I suspected that it had been

diluted, but Dr Shaw-Mackenzie's notes showed that it was made in the usual way, and there is no reason to believe that it has since been tampered with. It is evident, too, from the last column, that all these extracts are tryptic. Mellanby and Woolley [1914] state that glycerol extracts of pancreas are tryptic only on dilution. Shaw-Mackenzie [1915] has shown, by the serum plate method, that the *concentrated* extract is tryptic. I am able to confirm this, for, with the serum plate method, all our concentrated extracts are tryptic. Concentrated glycerol extracts of pancreas, therefore, can be kept for a considerable time and still retain lipoclastic and proteoclastic activity, the glycerol apparently inhibiting the destructive action of trypsin on lipase.

The stability of dilute extracts.

For most of our work the concentrated extract was diluted with twice its volume of water shortly before use. To what extent this dilute extract retains lipoclastic and proteoclastic activity after it has been kept for some time, has been the subject of further investigation.

The following preparations were made:

A.	Concentrated glycerol extract	...	67 cc.
	Distilled water	...	133 „
B.	Concentrated glycerol extract	...	67 „
	1 % solution of formaldehyde	...	10 „
	Distilled water	...	123 „
	(= 1 in 2000 formaldehyde)		

The lipoclastic and proteoclastic activities of these preparations were at once examined in the usual way. The flasks containing them were corked and left at room temperature for 5 days when a second examination was made. To A, from which 18 cc. in all had been removed, 2 cc. of toluene were added. Both flasks were corked and left at room temperature. Forty-six days after the date of preparation they were again examined. In all the examinations lipoclasia and proteoclasia went on for 24 hours in the incubator at 37°. The results are here given.

		A	B
Lipoclastic activity in cc. N/10 NaOH	{ at once	30.9	29.8
	{ after 5 days	30.1	29.1
	{ after 46 days	29.3	25.1
Tryptic activity in cc. N/10 NaOH	{ at once	5.1	4.8
	{ after 5 days	4.2	4.2
	{ after 46 days	3.2	3.3

The tryptic power of both had diminished to some extent, but in spite of the presence of trypsin both preparations were still strongly lipoclastic, the activity of the toluene preparation being practically unimpaired while that of the formaldehyde preparation was slightly diminished. The preparations gave no odour of putrefaction.

Even with greater strengths of formaldehyde the extracts retain both lipoclastic and proteoclastic activity.

The following preparations were made:

No.	Concentrated extract cc.	Distilled water cc.	1 % formalde- hyde solution cc.	Toluene cc.
I	100	200	—	—
II	100	200	—	4
III	100	170	30	—
IV	100	140	60	—

The strength of formaldehyde in III was 1 in 1000 and in IV 1 in 500.

The lipoclastic and proteoclastic activities were examined in the usual way, incubation going on for 22·5 hours at 37°. The flasks containing the preparations were corked and left at room temperature. After 42 days No. I contained a mould (*Penicillium glaucum*) but there was no odour of any kind, No. II gave the odour of toluene, and Nos. III and IV had an oily but not putrid odour.

In every case, as also in the last experiment, a precipitate had separated out and risen to the top. The mixture was therefore always shaken before samples were removed for examination.

The activities just after preparation and 42 days later are given.

		I	II	III	IV
Lipoclastic activity in cc. N/10 NaOH	at once	23·4	23·4	22·7	23·7
	after 42 days	23·4	28·9	16·4	13·1
Tryptic activity in cc. N/10 NaOH	at once	5·5	5·5	5·3	4·9
	after 42 days	5·1	5·3	5·0	4·6

All were still lipoclastic and proteoclastic. The proteoclastic activity showed no appreciable change. The lipoclastic activity was diminished in the extracts containing formaldehyde but gave a higher number in that containing toluene. The activity of the extract without antiseptic was in no way impaired. It is interesting to note that formaldehyde, in the strengths used, did not destroy the lipase.

Thus it appears that in these dilute extracts trypsin and lipase can exist together without destruction of the latter, the extracts containing enough glycerol to protect the lipase.

CONCLUSIONS.

1. Glycerol extracts of pancreas retain their lipoclastic and proteoclastic activity even after more than five years.

2. Dilute extracts (1 in 3), kept with or without antiseptics for six weeks, show both lipoclastic and proteoclastic activity.

3. Trypsin and lipase are present together in concentrated and dilute glycerol extracts, the glycerol apparently inhibiting the destructive action of trypsin on lipase.

I wish to thank Dr Rosenheim for suggesting to me the subject of this research and for much kind assistance.

REFERENCES.

- Bradley, H. C. (1909), *J. Biol. Chem.* **6**, 133.
Mellanby, J. and Woolley, V. J. (1914), *J. Physiol.* **48**, 287.
Rosenheim, O. and Shaw-Mackenzie, J. A. (1910), *J. Physiol.* **40**, *Proc.* xi.
Shaw-Mackenzie, J. A. (1915), *J. Physiol.* **49**, 216.
Sørensen, S. P. L. (1908), *Biochem. Zeitsch.* **7**, 45.
Terroine, E. F. (1910), *Biochem. Zeitsch.* **23**, 404.
Tsuji, K. (1915), *Biochem. J.* **9**, 53.
Umeda, N. (1915), *Biochem. J.* **9**, 38.