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Journal Physiology, Vol. XL.
[*From the Proceedings of the Physiological Society*, Feb. 19, 1910.]

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In the preceding communication it was mentioned that a strongly lipoclastic glycerin extract of pancreas was found to be inactive after careful filtration. This holds true for both the diluted and undiluted extract and agrees with most of the previous statements in literature. Owing to this behaviour the lipoclastic enzyme of the pancreas is now generally considered as an intracellular enzyme. No attempt seems to have been made to examine the fraction of the extract which remains behind on the filter, probably owing to its small amount and its uninviting physical properties.

The observation was made that on diluting a glycerin extract of pancreas with water and allowing the milky fluid to stand, a bulky white precipitate settled, whilst the supernatant fluid became perfectly clear. As the latter was found to be inactive, it was hoped to obtain the active enzyme from the precipitate.

To test this point a number of experiments were made on a small scale. Amounts of 5 c.c. of the diluted extract were filtered under pressure through a layer of paper pulp and a perfectly clear filtrate was obtained. Both filtrate and paper pulp with its contents were tested separately with regard to their lipoclastic action, a corresponding amount of unfiltered extract serving as a control. The somewhat surprising fact was found, that the insoluble residue was practically inactive. In order to avoid a possible inhibitory action of the paper pulp, the experiments were repeated by simply effecting the separation of the precipitate by means of the centrifuge. The same result was obtained.

On mixing, however, either the paper pulp residue or the centrifugalised deposit with the clear fluid the two previously inactive constituents of the mixture exerted the same lipoclastic power as the control unfiltered extract. This behaviour is clearly shown in the following experiment. 10 c.c. of glycerin extract were diluted with 40 c.c. water. 5 c.c. of the dilute extract were used for each experiment and allowed to act on 5 c.c. of olive oil emulsion for 18 hours at 37° C.

	c.c. $\frac{N}{10}$ KOH required
Dilute Extract	18·1
Centrifugalised Fluid[1]	2·8
Centrifugalised Deposit + 5 c.c. water[1]	1·0
Centrifugalised Fluid + Deposit	18·0

It was further found that the activity of the filtrate was not diminished by boiling, whilst the potential energy of the residue was destroyed by heating. See following experiment.

Centrifugalised Fluid + heated Deposit	3·1
Centrifugalised Deposit + heated Fluid	26·3
Uncentrifugalised Extract	26·1

It seems therefore that pancreatic lipase is a complex enzyme which can be separated into two inactive fractions by mere filtration through filter paper, a fact which has so far not been observed in any other enzyme. The only analogous cases recorded in literature are that of the esterase of the liver and that of yeast zymase. In the case of liver esterase Magnus separated his co-enzyme by dialysis, whilst in the second case Harden and Young obtained a separation by dialysis as well as by filtration through a Martin-gelatin filter. Following the not altogether satisfactory terminology now in general use, the term co-enzyme of pancreatic lipase is proposed for the substance contained in boiled aqueous or glycerin extracts of pancreas.

The inactive lipase can be easily obtained as a dry powder. Care was taken to remove the soluble proteoclastic enzymes completely by washing with water and fat as well as cholesterin-like substances by subsequent extraction with alcohol, aceton and ether. The yield of dry inactive lipase, free from proteoclastic enzymes, fat, etc., varied between 0·2 and 0·5 % of the glycerin extract (0·6 to 1·5 % of the organ).

In order to obtain a solution of the co-enzyme free from proteoclastic and other enzymes the clear supernatant fluid obtained as above was boiled and filtered. In order to keep it in a convenient form the solution was concentrated to a fifth of its volume. The co-enzyme may also be obtained directly from the pancreas by extraction with water and freed from enzymes and coagulable proteins by boiling and subsequent filtration.

[1] The slight activity of both fluid and deposit is probably due to the difficulty of their complete separation by this method.

Many experiments have shown that the activity of the mixture of inactive lipase and co-enzyme is as great as that of the original pancreatic extract and it can be demonstrated by using as little as 5 mg. o the dry powder mentioned above.

With regard to the chemical nature of the co-enzyme it may be stated that it dialyses readily, is thermo-stable and seems to be soluble in dilute, but not in absolute alcohol and insoluble in ether. Its activity is lost on incineration of the active dialysate.

This separation of lipase into co-enzyme and inactive lipase makes it possible to analyse the action of bile salts. The results of experiments, which will be communicated later, showed that sodium cholalate had no action on the co-enzyme, but possesses a powerful activating action on the insoluble lipase, in the course of which the latter is rendered soluble. The supposed activating effect of lecithin (and kephalin) was not confirmed, but it was found that one of the cleavage products of lecithin, namely glycerophosphoric acid (as a sodium salt), induces a remarkable activation. Choline as hydrochloride or as the free base, cannot act as an activator, but brings the insoluble lipase into solution, a fact which will be further examined in connection with the state of inactive lipase in pancreatic juice. Acids and alkalis were found to be inactive.

The effect of serum on pancreatic lipase, recorded in the preceding communication, made it desirable to investigate its action on inactive lipase. Both normal animal and human sera were found to activate the inactive lipase to a remarkable degree. Their effect is shown in the following table. The inactive lipase was used as a fine emulsion in water (1 c.c. containing 10 mgs.); 5 c.c. olive oil emulsion were used in each case.

	c.c. $\frac{N}{10}$ KOH required
Inactive Lipase (10 mgs.) + 6 c.c. Water	0·9
Co-enzyme Solution (5 c.c.) + 1 c.c. Water	0·9
Inactive Lipase + Co-enzyme + 1 c.c. Water	20·2
Inactive Lipase + 1 c.c. Human serum + 5 c.c. Water	29·2
Inactive Lipase + 1 c.c. Dog's serum + 5 c.c. Water	31·4

It will be seen that the activation by the serum is greater than that induced by the amount of co-enzyme used in this case. It was found that serum also activates the inactive lipoclastic enzyme of fresh pancreatic juice of the dog as obtained by the injection of secretin.

Pancreatic juice	Olive oil Emulsion	Water	Serum	c.c. $\frac{N}{10}$ KOH required
1 c.c.	5 c.c.	—	—	0·2
1 c.c.	5 c.c.	5 c.c.	—	2·0
1 c.c.	5 c.c.	4 c.c.	1 c.c.	39·6

These facts justify the assumption that the co-enzyme of pancreatic lipase is of the nature of a hormone, being carried away by the blood when produced in the pancreas. It may possibly play a *rôle* in enabling the lipases of the tissue to hydrolyse the fat carried to them by the blood-stream after digestion. In analogy to proteins and polysaccharides it may be assumed that the hydrolysis of fats precedes their oxidation or storage in the tissues and the existence of a lipoclastic co-enzyme in serum suggests its possible function in the process, so far unexplained, occurring during the disappearance of fat from blood.

It will also be interesting to study the effect of serum on inactive lipase in relation to hæmolysis, especially since it has been shown by Neuberg and others that the phenomena of hæmolysis are closely associated to those of lipolysis.