岡山醫學會雜誌第四百三十號
大正十四年十一月三十日發行
OKAYAMA-IGAKKAI-ZASSHI
Nr. 430, Nov. 30, 1925

原　　著

A Contribution to the Action of Pancreatic Lipase.

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Received for publication, July 29, 1925.

In accordance with the views of O. Rosenheim and E. F. Terroine on the studies of pancreatic lipase, it is acknowledged that the pancreatic lipase is consisted of two components: a thermolabile zymoid (Complemente) and a thermostable co-enzyme (ambozeptor), as that of toxin. Rosenheim isolated a thermostable co-enzyme from the pancreatic lipase and N. Umeda divided it into two parts; a soluble thermostable part and an inactive insoluble part from the glycerol extract of pancreas by ultrafiltration and proved a co-enzyme in it. De Jouge could make a pancreatic juice inactive and reactive and so agreed with the above view.

It is generally proved by various investigations of R. Magnus, v. Furth and Schutz and Donath etc., that the pancreatic lipase is principally secreted in the form of inartive zymogen but it is activated by the salts of the bile acids. It is, however, very doubtful whether the bile-acid, on this occasion, will substitute for a co-enzyme and transform the zymogen into a complete lipase, or it will act upon the complete ferment and strengthen its function simply as a catalyst. J. M. Mellanby viewed that the lipase in the glycerol

extract of the pancreas is originally insoluble and inactive but it is activated by the action of the bile acid. He illustrated this phenomenon by the fact that an insoluble component in the lipase becomes soluble as if it is seen in the pancreatic juice. However, the lipase is naturally soluble and so it can not consent to his view. R. Willstatter observed that the pancreatic lipase as well as the gastric lipase in the acid mediums is not activated by the action of the bile acid but the former's function is rather checked. He viewed that the salts of bile acids combined with the albumin in the pancreatic juice precipitate a colloidal substance and it adsorbs a substrate; the fat and lipase, and acts upon as a complex adsorbate. Consequently the function of the cholic acid is of use in the alkaline mediums. H. Donath concluded that by adding an inactive lipase which is inactivated by heating at 56 – 60°C to the active pancreatic lipase, its function is checked, especially a large quantity of the formers makes it completely functionless. However, if the existence of co-enzyme is in this case admitted, the function of the active lipase should be rather strengthened.

On the alcoholic fermentation of yeast, Harden und Young found the zymogen and co-enzyme decomposed, and pointed out that this co-enzyme is thermostable and dialysable. If so, the following two questions must be naturally dissolved: 1. when the pancreatic juice dialysed is added to the salts of bile acids, 2. when the pancreatic juice dialysed is added to the ferment which was inactivated by heating. These are very interesting problems to investigate not only the composition of the ferment but to give a certain definition to the bile acid influenced upon the pancreatic lipase.

Lastly, a little spaces will be here spared as to the action upon the pancreatic lipase to blood serum. Beizke and Neuberg proved that the function of steapsin to the castor-oil is strengthened its function by adding a normal rabbit's serum. Achard and Clerc remarked that inactivated lipase at 60—62°C is reactivated by adding fresh serum. Still, H. Donath reported that inactivated pancreatic lipase at 60 – 63°C is reactivated by adding horse-serum but inactivated ferment solution at 77 – 80°C is not restored its normal function even though the above serum is added, and stillmore, he noticed that dealbuminous serum has no power to regain the above function. It is questionable that whether or not the pancreatic lipase will be, on this occasion, inactivated at 60—63°C. In fact, Donath's experiment is shown that the lipase at 60—63°C has a function to decompose ester though it is very weak and he also evidently addmitted this fact. Consequently, it is doubtful whether the inactivated pancreatic lipase will be reactivated by the bood serum or the function of lipase once weakened is strengthened again or farthermore, the inactivated ferment may take part in strengthening the action of the serum

lipase as there always exists a little serum lipase in blood. If the above assumption that the reactivity of the pancreatic lipase is existed and also it is not reactivated by the dealbuminous serum, as Donath's view, is affirmed, then the proteine in the serum must be considered to function as a zymoid. If so, how it is resulted when the components of serum, for example, glycocol, creatin, creatinin, urea and serum albumin etc., are functioned on the inactive ferment? However, Willsttater illustrated from the standpoint of physical chemistry that the proteine in a serum adsorb both the lipase and substrat in the form of colloids and they easily function as an activator. The above various problems will be here further investigated. The steapsin preparation for this experiment was obtained from the glycerol extract of cattle's pancreas, and tributyrol was used for substrate. To make certain of the records all the tests were always repeated. The preparations were incubated at 37°C for three hours and the fatty acid uncombined was quantitatively determined by $n/10$NaOH solution after Kanitz's method and investigated comparatively their respective figures.

Experiment A.

Assuming that the ferment is, as above mentioned, composed of thermolabile and thermostabile components, the ferment was heated to some extent, then it is supposed that the above two components may be functionally quite different and consequently, the following question that how the above heated ferment will be influenced upon the fresh ferment must be investigated. The pancreatic juice divided into 5 groups were heated for 2 hours in the water bath at 100, 90, 80, 70°, and 60°C respectively. Take 1.0 cc of pancreatic juice and tributyrol respectively and adds water to make the total volume of the mixture 5 cc. (Notice: in the following all experiments, the tributyrol was always used in the above volume and the total volume of the mixture was made 5 cc by adding water.)

a indicates the test with heated pancreatic juice.
b indicates the test with fresh pancreatic juice.
c indicates the test with the mixture of heated and fresh pancreatic juice.
d indicates the test with fresh pancreatic juice at 70 and 60°C each and in this test only, 2 cc of fresh pancreatic juice were used.

	a	b	c	d		a	b	c	d
Experiment 1 (at 100°C)	0.9	6.5	7.5	—	Experiment 4 (at 70°C)	0.9	7.4	12.1	13.5
	0.9	6.7	7.6	—		0.9	7.9	13.0	14.3
Experiment 2 (at 90°C)	0.8	6.7	7.7	—		0.9	6.9	9.3	11.8
	0.9	6.6	7.5	—		1.0	7.0	9.6	12.2
Experiment 3 (at 80°C)	0.9	6.5	7.7	—		1.0	6.7	9.1	10.5
	0.9	6.9	7.9	—		0.9	6.8	8.4	11.1
					Experiment 5 (at 60°C)	4.3	7.1	9.4	10.5
						4.2	7.1	9.1	11.1

Referring to the above experiments, the pancreatic lipase at 70—100°C is entirely inactive and it is slightly active at 60°C. The pancreatic lipase at 80—100°C is indifferent from the fresh ferment but at 70°C it strengthens the action of the fresh ferment. This indicates that the two components of the ferment are entirely destroyed at 80°C and over, but at 70°C, the thermostabile component only still keeps its function. At the same time, the fact that the thermostabile component strengthens the action of the fresh pancreatic lipase can be assumed that this component is corresponding to the co-enzyme. Willstatter, however, viewed that the albumin in the pancreatic juice is on this occation functioned as an activator.

Experiment B.

In this series, it was tested that on centrifugalization of the heated pancreatic juice, whether or not the inactive fermet is precipitated. The pancreatic juice with water in ana was heated for 2 hours at 70°C, zentrifugalized and made a pap from the sediment deposited, being added water in ana. The pap (the sediment) and the centrifugalized part were each actioned to the fresh ferment.

a indicates the test with 2.0 cc of centrifugalized part.

b indicates the test with 2 0 cc of sediment.

c indicates the test with 1.0 cc of fresh ferment.

d indicates the test with 1 0 cc of fresh ferment and 2.0 cc of centrifugalized part.

e indicates the test with 1.0 cc of fresh ferment and 2.0 cc of sediment.

a	b	c	d	e
0.5	1.1	6.7	7.3	8.6
0.5	1.1	6 7	7.0	8.8

By the above result, it is well proved that on centrifugalization of the heated pancreatic juice, the inactive ferment is precipitated and this phenomenon is to be illustrated by absorption process.

Next, the results of the salts of bile acid actioned to the inactive pancreatic lipase at 70°C and also its centrifugalized part and sediment were observed as follows:—The pure cholic acid was neutralized with $n/10$NaOH solution and made 1% aqueous solution from it.

Experiment 1 indicates the test with inactive ferment at 70°C.

Experiment 2 indicates the test with centrifugalized part.

Experiment 3 indicates the test with sediment.

Marks a, b, c and d signify the test with 1% cholic acid solution and their quantities are 0.5, 1.0, 1.5, and 2.0 cc respectively.

	without acid	a	b	c	d
Experiment 1	1.0	1.0	1.0	1.0	1.0
	1.0	0.9	1.0	1.0	1.0
Experiment 2	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5
Experiment 3	0.7	0.7	0.7	0.7	0.6
	0.7	0.75	0.6	0.7	0.7

The above experiment remarks that the salts of bile acid is entirely indifferent from the function of the inactive ferment and also of its centrifugalized part and sediment.

Experiment C.

There are some differences between the functions of pancreatic lipase with and without the salts of bile acid and its each rapidity was here comparatively investigated.

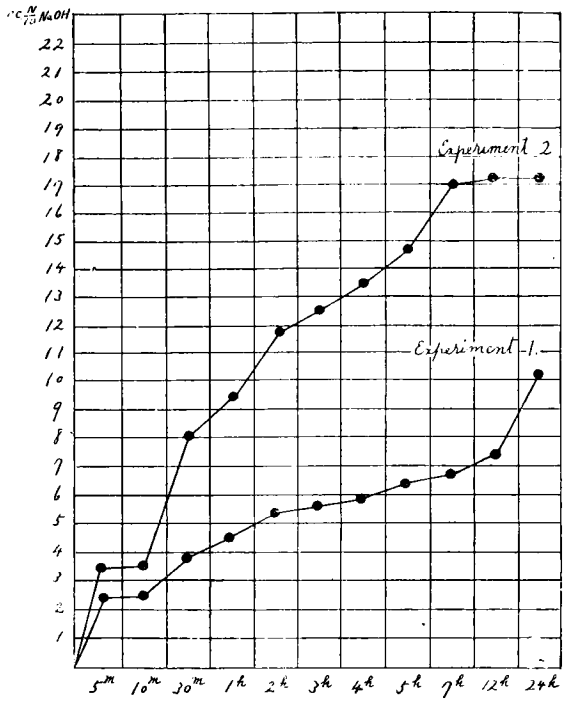
Experiment 1, case without cholic acid.

Experiment 2, case with 1.0 cc of 1% cholic acid.

1.0 cc of pancreatic juice and tributyrol were each kept in incubator for from 5 minutes to 24 hours and examined in various intermittences as follows:—

	5 m.	10 m.	30 m.	1 h.	2 h.	3 h.	4 h.	5 h.	7 h.	12 h.	24 h.
Experiment 1	2.4	2.4	3.9	4.4	5.3	5.6	5.8	6.2	6.6	7.2	9.2
	2.4	2.4	3.9	4.4	5.3	5.7	5.7	6.9	6.5	7.2	9.1
Experiment 2	3.4	3.6	8.2	9.7	11.8	12.6	13.1	14.4	16.9	17.1	17.1
	3.6	3.5	7.8	9.2	11.9	12.0	13.6	14.8	17.0	17.2	17.3

Considering the above experiment, the function of lipase without the salts of bile acid is very gradually heightened. The function of lipase with the salts of bile acid is, however, markedly heightened and at the 7th hour it reaches to the maximal point and at the 12th and 24th hour, it keeps the highest point. It is well shown in the following graph. H. Donath applicated the cholic acid in various densities to the pancreatic lipase and observed that the function of lipase gradually increased reaches to the maximal point at certain density and that no more increased its function even the density of the cholic acid is far more heightened. He illustrated it by the reason that the zymoid is wholly activated by the cholic acid, viz., there is no more zymoid stored to be activated by the cholic acid. Willstatter announced that it transfers to the acid mediums by the fatty acid and this fatty acid is to be considered to action as a paralysator of the lipase.



Experiment D.

It is said that the co-enzyme in the yeast juice is thermostable and dialysable as above mentioned. If so, how will the function be changed when the pancreatic juice is dialyzed? In this series, this was investigated. By means of collodion sac, it was dialyzed daily from one to 7 days and its result is as follows:—

a indicates the test with 1.0 cc of pancreatic juice.

b indicates the test with 1.0 cc of boiled pancreatic juice.

	Before dialy.		1th day.		2th day.		3th day.	
	a	b	a	b	a	b	a	b
Experiment 1.	6.9	1.0	4.0	0.6	3.4	0.4	1.5	0.2
Experiment 2.	6 8	0.9	2.7	0.3	2.0	0.2	1.8	0.1
	4th day.		5th day.		6th day.		7th day.	
	a	b	a	b	a	b	a	b
Experiment 1.	1 1	0.2	1.1	0.2	1.0	0.15	1.1	0.1
Experiment 2.	1.1	0.15	1.1	0.15	1.5	0.1	1.5	0.1

By this test, it is acknowledged that the dialysation markedly decreases the function of lipase in the 1st day and the acidity of the pancreatic juice is at the same time lowered. On the 7th day, its power still decreases from 1/5 to 1/6 of the 1st day's. Then, the pancreatic juice which was dialyzed continuously for 7 days was functioned to both the cholic acid and inactive ferment at 70°C and its result is as follows:—

a indicates the test with 1.0 cc of boiled dialyzed pancreatic juice.

b indicates the test with 1.0 cc of dialyzed pancreatic juice.

c indicates the test with 1.0 cc of dialyzed pancreatic juice and 1.0 cc of 1% cholic acid.

d indicates the test with 1.0 cc of dialyzed pancreatic juice and 1.0 cc of inactive ferment at 70°C.

e indicates the test with 1.0 cc of inactive ferment.

a	b	c	d	e
0.1	1.7	5.2	3.1	0.9
0.1	1.7	5 3	3.1	0.9

The result tells that the pancreatic juice which was markedly decreased its function by the dialysation, is reactivated by both the cholic acid and inactive ferment at 70°C, and the former is particularly stronger than the latter.

Experiment E.

Referring to the reactivity of the pancreatic lipase due to the blood serum as above mentioned, H. Donath viewed that the inactive ferment at 60 to 63°C is reactivated by blood serum but at 77 to 80°C, it is vice versa. On the contrary, according to the result of experiment A, the function of lipase at 60°C is not inactivated though it becomes very weak, and consequently, the above result is doubtful as previously mentioned, whether it is reactivated by adding blood serum or it comes from the serum lipase strengthened. Therefore, it is, on this occasion, rather rightly to replace the inactive ferment at 70°C instead of at 60°C. A certain amount of blood obtained from the blood vessels of rabbit's ear lobe was centrifugalyzed and the function of lipase of the serum was examined.

a indicates the test with 1.0 cc of boiled serum. b indicates the test with 1.0 cc of fresh serum.

a 0 3 0.3 b 1.3 1.3

This examination proved that the function of serum lipase is very weak, comparing with that of the pancreatic lipase.

The result of 1.0 cc of blood serum with the cholic acid and inactive ferment at 70°C is as follows:—

a indicates the test with 1.0 cc of 1% cholic acid.

b indicates the test with 1.0 cc of inactive ferment at 70°C.

c indicates the test with only 1.0 cc of inactive ferment at 70°C without serum.

a 1.3 1.3 b 3.1 3.2 c 0.9 0.9

This result shows that the cholic acid is quite indifferent from the serum lipase. Contrarily, the inactive ferment at 70°C increases the decomposing power of ester by adding blood serum.

According to H. Donath's view, in this case, the inactive ferment is not reactivated by adding dealbuminous serum. Next, the result of the ingredient of the serum action-

ed to the inactive ferment was observed.

To the inactive ferment at 70°C, glycocol, creatin, creatinin, urea, uric acid, serum albumin and the white of eggs were respectively added and its result is as follows:—Amoung the above substances, those which belong to the acid group were previously neutralized with $n/10$NaOH solution and 1.0 cc of 0.1% solution was each used. The acidity resulted by the inactive ferment at 70°C only is always estimated 0.9.

glycocol	1.3	1.3	creatin	1.4	1.4	creatinin	1.5	1.5	urea	1.2	1.2
uric acid	0.9	0.9	albumin	1.5	1.5	white of egg	1.4	1.4			

This experiment proved that the serum albumin as well as the ingredient of the serum except uric acid, reactivate the inactive lipase. In this series, it was well understood that the increasing of the decomposing power of ester of the inactive ferment by adding blood serum is not due to the increasing of the function of serum lipase but comes from the reactivity of the inactive ferment, and still, that the ingredient of the serum is actioned as an activator to the pancreatic lipase.

Summary.

The ferment inactivated at 70°C strengthens the action of both the fresh and dialyzed lipase and is reactivated by the ingredient of the serum. By centrifugalyzation, the inactive ferment is remained in its sediment. The cholic acid is entirely indifferent from both the inactive ferment at 70°C and serum lipase, but it reactivates the dialyzed pancreatic lipase. The function of the fresh pancreatic lipase reaches to the maximal point at certain period by adding cholic acid and thereafter its permanent effect is settled.

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