

A NEW METHOD FOR SERUM LIPASE DETERMINATION AND SERUM LIPASE ACTIVITY IN LIVER DISEASES DETERMINED BY THIS METHOD

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SUMMARY

- 1) A new procedure for serum lipase determinations is presented. It is specific and sensitive. The incubation period is only 1 hour.
- 2) This method was clinically applied to some patients with acute pancreatitis and was found to be more useful than other commonly performed pancreatic function tests.
- 3) Serum lipase activity was determined in the patients with liver diseases consisting of acute hepatitis, chronic hepatitis, liver cirrhosis and hepatoma, and with biliary diseases. Serum lipase level was elevated in many of them.
- 4) In order to determine the mechanism of the hyperlipasemia, several investigations were performed. It was concluded that hyperlipasemia in patients with hepatic diseases might be due to abnormal pancreas function influenced by liver impairments.

INTRODUCTION

The measurement of serum lipase activity has been of significant clinical value since shortly after the introduction of a practical method by Cherry and Crandall in 1932¹⁾. This value has been lessened by the necessity for incubation for 24 hr. as well as by other difficulties inherent in the method. The numerous attempts have been made to overcome the limitations in the serum lipase determination. These modifications include the substitution of tributyrin²⁾ for olive oil; the reduction of incubation time³⁾; a turbidimetric method employing a very delicate substrate⁴⁾; the use of accelerators such as EDTA⁵⁾, albumin⁶⁾, Ca⁺⁺⁷⁾ and bile salts⁴⁾. These modified methods have other technical requirements.

In recent years several methods for the determination of small amounts of fatty acids in serum have been devised. Titration of free fatty acids⁸⁾, the most widely used method, is difficult to perform if only small amounts of biological material are available. A colorimetric method for long chain fatty acids was devised by Ayers⁹⁾, and modified by Iwayama¹⁰⁾ and Duncombe¹¹⁾.

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We applied this method to the measurement of fatty acids released by the hydrolysis of triglycerides by lipase. It is our belief that the serum lipase, when determined by the method proposed here, is accurate and reproducible, it can be performed in a short time (1 hr), and it is of greater value to the physician than the serum amylase determination.

Clinical experience has shown that elevated value for serum lipase is not pathognomonic of intrinsic pancreatic diseases and such hyperlipasemia has been reported in some cases of hepatic diseases¹²⁾. The fact that elevations of serum lipase may occur in liver diseases, however, does not seem to be generally appreciated. This is mainly due to the methodological difficulties of serum lipase determination.

We checked the serum lipase activity in liver diseases and in biliary diseases by our proposed method and discussed the mechanism of hyperlipasemia in hepato-biliary diseases.

EXPERIMENTAL METHODS AND RESULTS

I. A new rapid method for the determination of lipase activity and effects of several factors on this method

a) Method

Reagents

The copper reagent (Iwayama¹⁰⁾, partly modified)

aq. 1 M triethanolamine 9 vol

1 N acetic acid 1 vol

5% Cu (NO₃)₂·3H₂O 10 vol

Diethyldithiocarbamate reagent (Duncombe¹¹⁾) 0.1% (W/V) solution of sodium diethyl-dithiocarbamate in *n*-butanol.

Tris buffer (0.05 M pH 8.0) including 0.5% Sodium taurocholate.

Substrate

Lipomul (Corn oil 10 g/15 ml; 66.7% from Upjohn Co.,) 1 ml of commercial product was diluted with water to 30 ml.

Standard Solution of fatty acid

The chloroform solution of stearic acid (1.0 μEq/ml).

Procedure

i) Incubation system

0.05 M Tris-buffer (pH 8.0) containing sodium taurocholate (0.5%)
1.0 ml

Substrate 1.0 ml

Sample (Serum etc.) 0.5 ml

(1) 1.0 ml of the incubation mixture was taken as the control right after the addition of sample to the system.

(2) After the incubation for 1 hour at 37°C, 1.0 ml of the mixture was also

taken for the determination of liberated FFA (Free fatty acids).

ii) Colorimetric micro-determination of fatty acids

The control mixture or the incubated mixture stated as above [(1) or (2)] was placed in a 10-15 ml stoppered centrifuge tube with 5.0 ml chloroform and 2.0 ml copper reagent. The tube was stoppered and shaken vigorously for at least 2 minutes. It was then centrifuged for a few minutes to separate the phases cleanly and the upper phase was aspirated by a fine hypodermic needle. 2.0 ml of chloroform portion was pipetted into a clean dry tube. Then 2.0 ml of Carbamate reagent was added to this tube. After the solution was mixed, the extinction was read at 420 $m\mu$ against a blank solution which was prepared by adding 0.5 ml of water to stoppered centrifuge tube instead of (1) or (2).

The standard solution was subjected to the same procedure. The unit of lipase activity was calculated from the following equation. (2) - (1) FFA $\mu\text{Eq/ml/hr}$

b) The effects of several factors on this method

i) The relation of fatty acid concentration and extinction

Fig. 1 shows the relationship between the extinction and the concentration for a number of stearic acid standards. We could find a linear correlation.

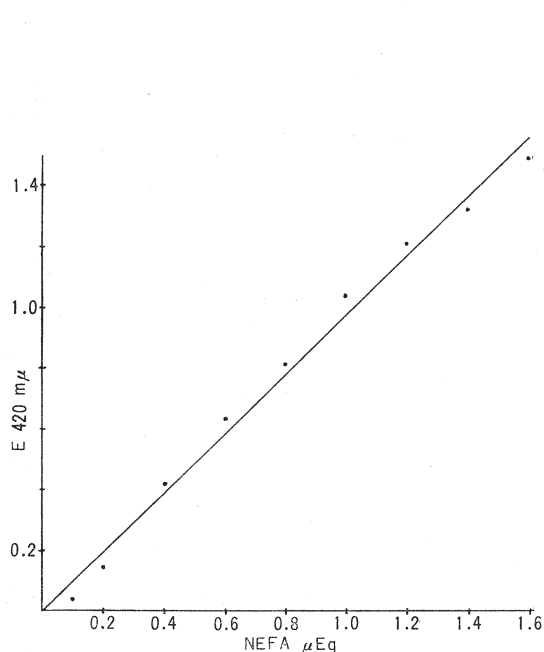


FIG. 1. Relationship between extinction and concentration for solutions of stearic acid in chloroform.

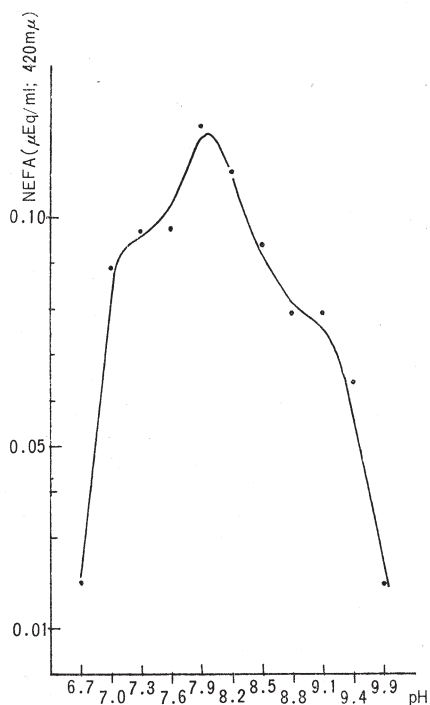


FIG. 2. Influence of pH.

ii) The effect of pH (Fig. 2)

The effect of pH in the incubation medium on lipase activity was investigated. The pH of Tris-buffer solution in the medium was changed from 6.7 to 9.9 and found the optimal pH of 7.9 to 8.2.

iii) Optimal wave-length of colorimeter

Using 0.5 μ Eq of fatty acid, we investigated the optimal wave-length of colorimeter.

Fig. 3 shows that it stands about 420 $m\mu$.

iv) Selection of the substrate

Olive oil and coconut oil emulsified with gum acacia or monoglycerides have been generally accepted as substrates for lipase. However, due to the deterioration with time and difficulty with preparation, Lipomul, an artificial triglyceride emulsion, was used as a substrate. This could be used successfully as a substrate for lipase giving approximately 1.5 fold greater activity than other substrates.

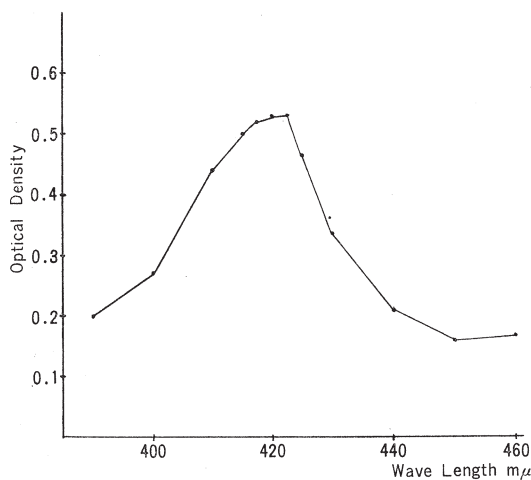


FIG. 3. Wave length of colorimeter (0.5 μ Eq).

TABLE 1. The effect of bile acid upon lipase activity*

	before	after	difference
Pancreatic Juice (1000×)			
Tris Buffer, only	242	274	32
1% Taurocholate added	2480	2560	80
Pancreas tissue of dog			
Tris Buffer, only	238	348	110
1% Taurocholate added	2530	2780	250

* Value of NEFA by Colorimeter.

v) The effect of taurocholate

Table 1 shows that the addition of 1% sodium taurocholate to the incubation medium stimulated the activity. But 0.5% sodium taurocholate was added for practical purposes, because the forming of copper taurocholate elevated the level of the control.

vi) The effect of incubation-time

We observed that there was a close relation between the lipase activity and the incubation-time, to 5 hours, as shown in Fig. 4. Then it was usually sufficient to determine the lipase activity by one hour's incubation.

vii) The difference by double determinations

The difference by double determinations was relatively slight.

Table 2 shows that the difference by the determination in 5 samples was 0.026 to 0.050 $\mu\text{Eq/ml/hr}$.

II. Serum lipase by the proposed method and Cherry-Crandall's method, serum amylase and urinary amylase in cases of acute pancreatitis

A series of serum lipase and amylase, etc. in some cases of acute pancreatitis was checked.

Fig. 5 shows a case in which serum lipase by this method and Cherry-Crandall's¹⁾ and serum amylase were determined in series. Serum amylase was determined by Somogyi's. The normal value by the proposed method was $0.078 \mu\text{Eq/ml/hr} \pm 0.0447$ (mean \pm S.D.).

In this case, serum lipase and amylase were increased simultaneously. The elevation of serum lipase activity by the proposed method was more distinct than by Cherry-Crandall's.

Serum amylase and lipase were reduced to the normal level in the 5th

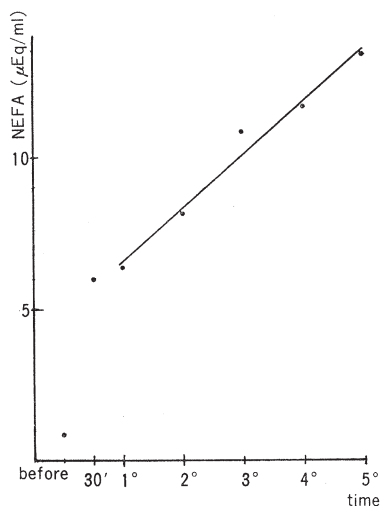


FIG. 4. The effect of incubation time upon the lipase activity.

TABLE 2. The difference by double determinations

($\mu\text{Eq/ml/hr}$)

Samples	The first determination	The second determination	Difference
S. A.	0.239	0.210	0.029
Y. S.	0.161	0.192	0.031
K. U.	0.111	0.161	0.051
T. H.	0.152	0.121	0.031
Y. O.	0.087	0.061	0.026

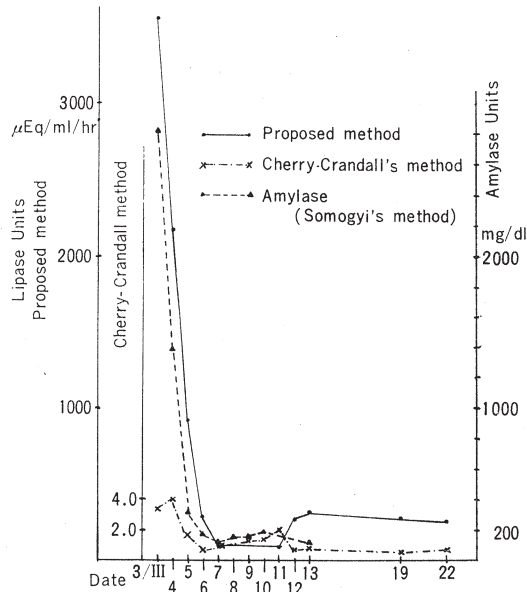


FIG. 5. Alteration of serum lipase activity in a patient with acute pancreatitis (34 years old man).

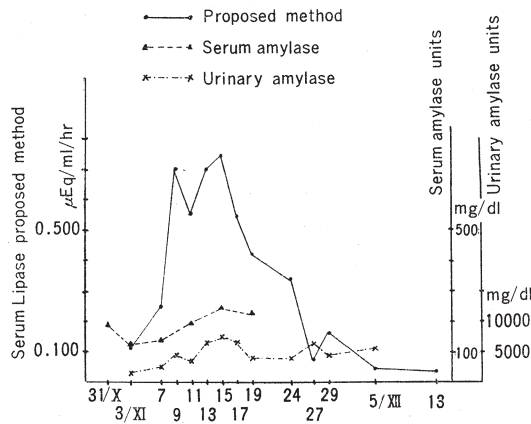


FIG. 6. Alteration of serum lipase activity in a patient with acute pancreatitis (43 years old man).

hospital day.

Fig. 6 shows a case in which serum lipase by this method, serum amylase and urinary amylase were checked in series. Urinary amylase was determined by the method which had been reported from our laboratory¹³⁾.

In this case serum amylase was elevated first, and then serum lipase was elevated. The elevation of urinary amylase was later.

The elevation of serum lipase activity was the most dominant, and this case was definitely diagnosed as acute pancreatitis by this method.

III. Serum lipase activity by this method in liver diseases

Clinical investigation

The serum lipase activity of 63 cases of hepatic and biliary diseases consisting of 10 cases of acute hepatitis (partly determined in series), of 16 cases of chronic hepatitis, of 18 cases of liver cirrhosis, of 5 cases of biliary diseases, were determined, and the correlation between the serum lipase activity and other commonly performed liver function tests was discussed with 16 cases of normal subjects for comparison.

These were diagnosed by clinical symptoms, liver function test, peritoneoscopy and liver biopsy. The serum lipase activity in these subjects is shown in Fig. 7.

The upper limit of the normals in this method was $0.168 \mu\text{Eq/ml/hr}$ (mean $+2$ S.D.).

i) Acute hepatitis

The determination was done in the icteric stage and in the convalescent stage. Four of 8 cases in icteric stage and 2 of 6 cases in convalescent stage showed hyperlipasemia. The abnormal rate and the mean value of the serum lipase activity are shown in Table 3. The elevation of the activity in the

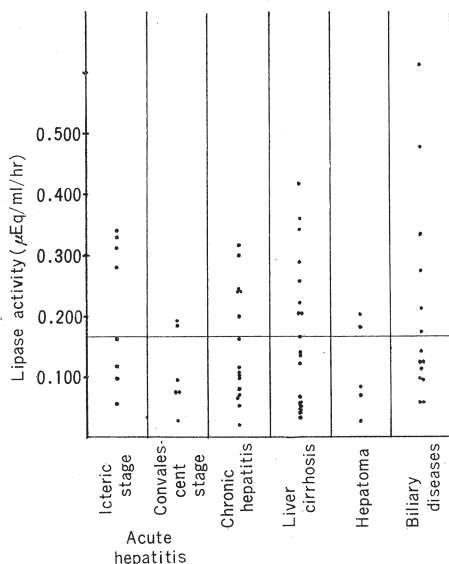


FIG. 7. Serum lipase activity by the proposed method in liver diseases.

The horizontal line shows the normal limit, $0.168 \mu\text{Eq/ml/hr}$ (mean $+2$ S.D.).

TABLE 3

	Abnormal Rate (%)	Mean \pm S. D.
Acute Hepatitis	50	0.212 \pm 0.116
Icteric stage		
Convalescent stage	33	0.109 \pm 0.066
Chronic Hepatitis	38	0.152 \pm 0.093
Liver Cirrhosis	44	0.176 \pm 0.120
Hepatoma	20	0.114 \pm 0.075
Biliary Diseases	43	0.207 \pm 0.166

TABLE 4. Abnormal rate of serum lipase in cases of normal or abnormal liver function tests

	Acute Hepatitis (%)	Chronic Hepatitis (%)	Liver Cirrhosis (%)	Hepatoma (%)	Biliary Diseases (%)
S-GOT abnormal	47	38	64	50	50
S-GOT normal	0	38	29	0	37
S-GPT abnormal	35	40	50	0	60
S-GPT normal	33	33	38	40	33
Total Bililubin abnormal	37	25	56	100	25
Total Bililubin normal	25	42	38	25	67
Al-Phos. abnormal	45	40	57	33	44
Al-Phos. normal	25	36	44	0	40
A/G abnormal	56	29	50	50	57
A/G normal	13	44	50	33	40
TTT abnormal	44	33	50	0	100
TTT normal	0	25	43	0	39

icteric stage had the significant difference as compared with that of the normals ($P < 0.05$).

No correlation could be made between the serum lipase activity and commonly performed liver function tests, but in cases showing abnormal values of S-GOT, A/G and TTT, the serum lipase activity was increased (Table 4). With recovery, the activity of serum lipase in these patients was reduced to the normal range.

ii) Chronic hepatitis

Six of 16 cases showed hyperlipasemia. The abnormal rate of the serum lipase activity in chronic hepatitis was 38%. The mean value had the sig-

nificant difference ($P < 0.05$) compared with that of the normals (Table 3).

No correlation could be made between the serum lipase activity and liver function tests. Each case showing abnormal liver function tests had not usually high lipase activity (Table 4).

iii) Liver cirrhosis

Serum lipase activity was found to be elevated in 9 of 18 cases. The abnormal rate and the value of the serum lipase activity in cirrhosis were higher than those in chronic hepatitis (Table 3). The mean value had the significant difference opposite to the normals ($P < 0.05$).

No correlation could be made between the serum lipase activity and other liver function tests in cirrhosis. However, in some cases showing high S-GOT value, the serum lipase was found to be elevated (Table 4).

iv) Hepatic carcinoma (primary and secondary)

In the rather small series of cases, evident elevation could not be found.

v) Biliary diseases

Six of 14 cases showed hyperlipasemia. The abnormal rate of the serum lipase activity was high, and the mean value had the significant difference ($P < 0.05$) as shown in Table 3.

No correlation could be made between the serum lipase activity and liver function tests. In many cases of hyperlipasemia, serum bilirubin was found to be normal. In these cases, however, S-GOT, S-GPT, A/G and TTT indicating liver impairments showed abnormal values.

IV. Gel filtration applied to the study of lipases

In order to determine the mechanism of hyperlipasemia in hepato-biliary diseases, the following investigations were performed. Several materials were fractionated by gel filtration to study the composition of lipase activity and to know which component was intensified in pancreatic or hepatic impairment.

a) Materials and Methods

The following materials were used for the experiments; pancreatic juice derived from drainage fluid of a healthy untreated dog, serum of a healthy dog and a dog with acute pancreatitis induced by the injection of 10% aq. bile into pancreatic duct on the 7th day, human serum of the healthy person and the person with hepatic impairment, the liver of a rat (prepared by homogenizing 5 g of liver in 10 ml saline, then centrifuging the mixture and separating the supernatant). The determination of the lipase activity was performed by the proposed method.

b) Gel filtration¹⁴⁾

Human serum, dog serum, and pancreatic juice were filtrated with Sephadex G=200, and rat liver with Sephadex G=100.

Each Sephadex was allowed to swell in saline before use. Columns (60 ×

2.5 cm) were packed in the cold with the swollen gels as described by Flodin. Samples were applied to the tops of the columns. Flow rates were about 20 ml/hr. Effluents were collected in 5 ml fractions. All columns were run at 0-5°C.

c) Results

i) Dog pancreatic juice

A large and single peak appeared about in fraction tube No. 43, as shown in Fig. 8. This elution pattern corresponded almost to the part of protein measured by Lowry's method.

ii) Serum of a untreated dog (Fig. 9)

The elution diagram showed a large peak around tube No. 22 and followed

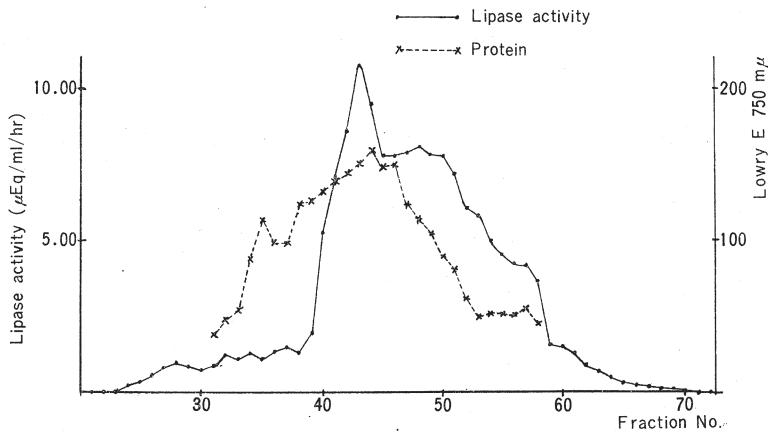


FIG. 8. Pancreatic juice lipase activity pattern of a normal dog.

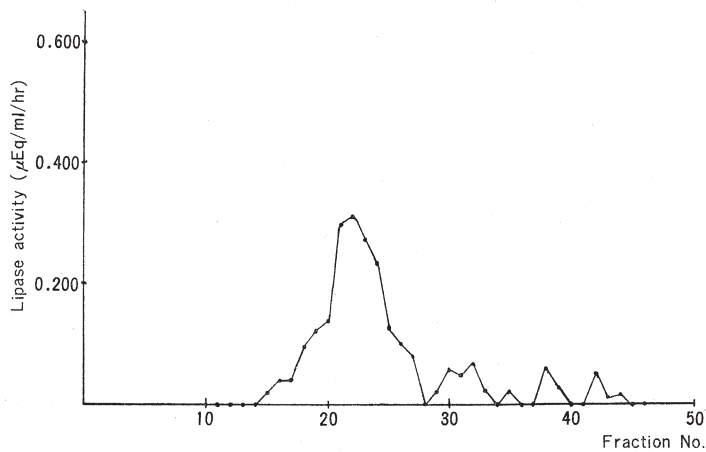


FIG. 9. Serum lipase activity pattern of a normal dog.

by several very small peaks.

iii) Dog pancreatitis serum on the 7th day

The elution diagram showed a large peak about at the same site as in the untreated. The activity was much higher. Several very small peaks also appeared later (Fig. 10).

iv) Healthy human normal serum (Fig. 11)

A large peak appeared around tube No. 23, and this peak seemed to be

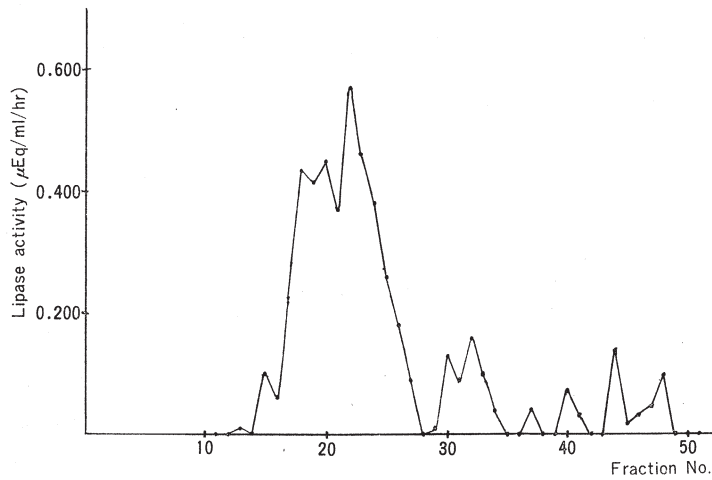


FIG. 10. Serum lipase activity pattern of the dog with acute pancreatitis (7 days after induced pancreatic impairment).

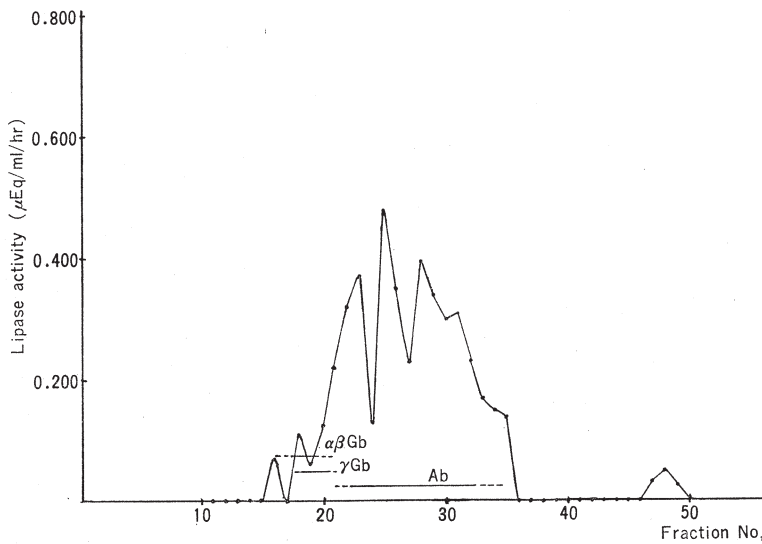


FIG. 11. Normal human serum lipase activity pattern,

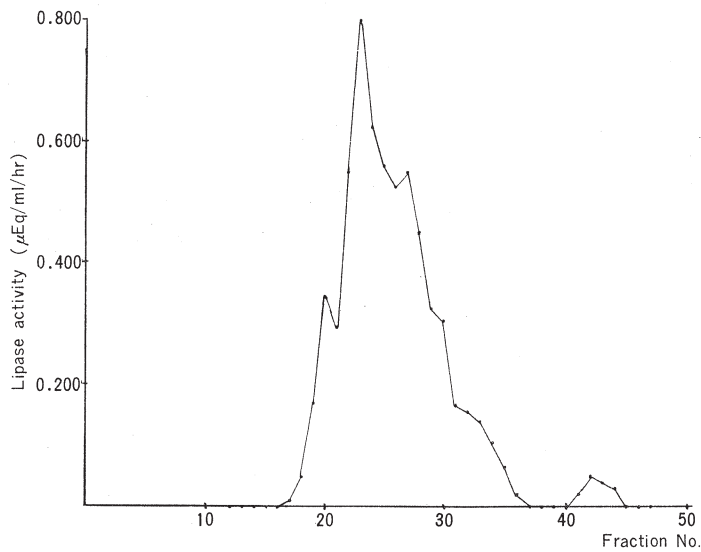


FIG. 12. Serum lipase activity pattern of human hepatic impairment.

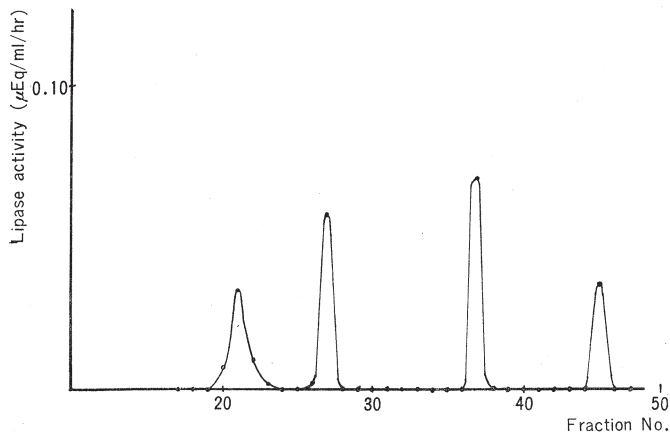


FIG. 13. Lipase activity pattern in rat liver

separated in two parts. This peak corresponded almost to serum proteins especially to serum albumin on electrophoresis. A very small peak appeared later.

v) Human serum in hepatic impairment

The main large peak appeared around the same site as in the normal, but the activity was much higher. Several very small peaks appeared later (Fig. 12).

vi) Rat liver (Fig. 13)

Although the elution pattern showed four peaks, each activity was very low.

V. Pancreas function in hepato-biliary diseases

In some cases of hepato-biliary diseases, as stated above, several pancreas

function tests were performed.

a) Pancreozymin-secretin test

Pancreozymin-secretin test¹⁵⁾ was performed in 14 cases composed of 7 cases of hepatic diseases and of 7 cases of biliary diseases. Three factors (volume, amylase output, and bicarbonate) were checked.

All factors were abnormal in one case of hepatic and of biliary diseases. One of them was abnormal in one case of hepatic diseases and in two cases of biliary diseases. Serum lipase activity was elevated in these cases.

b) Serum amylase

Serum amylase by Somogyi's method was performed in 11 cases composed of 8 cases of hepatic diseases and of 3 cases of biliary diseases. An abnormal value was not found in any of them.

c) Urinary amylase

The urinary amylase activity was determined in 6 cases. An abnormal value was observed in one case of hepatic diseases.

DISCUSSION

Although the clinical usefulness of lipase determinations has been well established, the test is not widely used. This may be due to following factors: the long time required, the methodological difficulties, and the low sensitivity of their procedures. Attempt was made to improve the method of lipase determination. This rapid procedure is based on Duncombe's colorimetric micro-determination of long chain fatty acids¹¹⁾.

In order to determine lipase activity there have been three ways. The one and the second are based on determining free fatty acids or glycerol⁶⁾ released from triglycerides, and the third is the turbidity method⁴⁾. In general, the determination of lipase activity is performed by measuring free fatty acids. Commonly used Cherry-Crandall's method is also based on this principle, however, this method requires long time, and the emulsified substrate is separated.

Then various methods for free fatty acid determination have been devised. The most widely-used method is that of Dole⁸⁾, a titration method which has been introduced into the lipase determination. But titration of weak acids is not simple, and interference might be caused by phospholipids and lactic acid, etc. A colorimetric method for long-chain fatty acids was devised by Ayers⁹⁾, and modified by Iwayama¹⁰⁾ and Duncombe¹¹⁾. Modifying this method, we applied it to the lipase determination. In our methods several factors were discussed.

The straight line obtained with stearic acid could be used as a calibration line because lines obtained with several fatty acids are fairly close together. The right state for lipase action apparently exists in emulsions prepared with

long chain triglycerides¹⁶⁾. The emulsifier may be gum arabic¹⁷⁾, gum acacia¹⁾, or monoglycerides¹⁸⁾. In order to avoid the difficulties of substrate preparation and to stabilize substrate contents, we used Lipomul, an artificial triglyceride emulsion as the substrate. It has been suggested that the activity is potentiated by serum albumin⁶⁾, $\text{Ca}^{++7)$ and taurocholate⁴⁾. There are some opinions opposed to these¹⁹⁾. In our investigation the activity was potentiated by Sodium taurocholate.

Serum lipase was found to have an optimum hydrolysis when using a buffer with a pH of 7.9 to 8.2.

The best advantage of our method was the short incubation period. One hour's incubation was usually enough.

This method was clinically applied to some cases of pancreatic diseases and was found to have more advantages in simplicity and sensitivity than other methods.

Hyperlipasemia has been reported not only in patients with pancreatic diseases but also in patients with hepatic diseases¹²⁾. However, only a few instances have been reported, results have been various and the mechanism of hyperlipasemia has not been clear. These are mainly due to the methodological difficulties of the determination.

In our clinical experiments, composed of 63 cases of hepato-biliary diseases, serum lipase was determined. Although in no case was the elevation very marked, serum lipase was found to be elevated in many cases. Fifty per cent of patients in the icteric stage of acute hepatitis, 38% of patients with chronic hepatitis, and 44% of patients with liver cirrhosis showed hyperlipasemia. In many cases of biliary diseases, especially with abnormal liver function tests, serum lipase was elevated. Namely many patients with liver impairments were found to have hyperlipasemia. Although its mechanism has not yet been known, Cummins, *et al.*¹²⁾ suggest three possibilities. The serum lipase may be elevated due to actual production of the enzyme in degenerating liver tissue. Another possibility is that the liver normally regulates the blood enzyme level by removing any excess. A third possibility is that hepatic diseases may be associated with an accompanying pancreatitis.

In fact, recently, liver lipase was found⁶⁾²⁰⁾. However, it was unlikely that the elevation of serum lipase in hepatic diseases was directly due to the liver lipase. No correlation could be made between hyperlipasemia and commonly performed liver function tests including serum transaminase in our experiments.

The study using Sephadex suggested that serum lipase was mainly due to pancreas and that the elevation of serum lipase in hepatic diseases was found to be perhaps due to the pancreatic portion. Although useful pancreas function tests are still very few, we performed some of them including Pancreozymin-secretin test in cases of hepato-biliary disease as stated above. Some of them

showed abnormal pancreas function. Recently Forell, *et al.*²¹⁾ showed the abnormality of Pancreozymin-secretin test in liver diseases as well.

In fact, it has been reported that post-mortem examination of many patients with hepatic diseases reveals morphological changes of pancreas. These are classified in two groups²²⁾²³⁾; degeneration or necrosis of parenchymal cells in pancreas, changes of mesenchyma in pancreas.

We may be able to conclude that hyperlipasemia in patients with hepatic diseases was due to abnormal pancreas function influenced by liver impairments. In order to determine how the pancreas will be influenced by liver impairments, further investigations are required.

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