Relation Between Fat Content and Lipase Activity in Liver.
An Analytical and Histochemical Study.

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There has been few histochemical study of enzymes with quantitative analytical study of corresponding substrative substance, while many histochemical studies have been published.

The authors intended to analyze the relation between lipase activity and fat content in liver, and histochemical and quantitative analytical studies were performed. The results obtained will be presented here.

Materials and Methods

Twenty four autopsy cases of our department were examined. The items of autopsy cases were as follows; 7 cases of cirrhosis of liver, 2 cases of fatty liver, each one case of hepatitis, congenital atresia of bile duct and liver cell carcinoma (hepatoma), and 12 cases without noticeable liver disease. Livers of experimental animals which were yellow rice fed mice, choline deficient diet fed rats and colchitin administered rats were also investigated.

Fresh livers were fixed in cold aceton and paraffin sections were prepared as usual manner. Tween 20, 40, 60, 80, and linolenic acid methyl ester were used as substrate for Gomori's lipase stain. Tissue sections were incubated in those substrates for 24 hours, at 37°C. Frozen sections of formalin fixed tissue were stained with Sudan III.

Qualitative and quantitative analysis of fat constituents in fresh livers were performed by means of infra-red spectrophotometry, gas chromatography and chemical analysis.

Results

I. Fat content in liver.

Chemical analysis of the livers revealed that the average fat content in control (normal) livers was about 3.5 g per 100g wet weight. On liver lipid fraction of human and experimental fatty liver it was recognized that neutral
fat fraction increased remarkably in amount, and cholesterol, especially its esterified form, showed a tendency to increase. On the contrary, phospholipids decreased remarkably which was due to decreased amount of lecithine (Fig. 1). Infra-red spectrophotometrical survey revealed the same results described above (Fig. 2).

Fatty acid fractions were studied by means of gas chromatography. The results obtained are shown schematically in Figure 3. In fatty liver, increased amount of myristic and oleic acids and decreased amount of stearic and palmitic acids were recognized.

The fact that the amount of sudanophilic droplets in frozen sections, which generally assumed as neutral fat, being proportionate to the neutral fat content in liver was convinced by the comparative survey of chemical and histopathological studies.

II. Relation between lipase activity and fat content in liver.

The histochemically demonstrable lipase activity in liver seemed to be preserved fairly well even more than 10 hours after death.

The distribution of histochemically demonstrable lipase was essentially the same among the used substrates. According to the difference of substrate, however, the intensity of histochemically demonstrable lipase was somewhat different and following sequence was recognized ; Tween 20 > Tween 40 = Tween 60 > Linolenic acid > Tween 80.

An opposite distribution between sudanophilic droplets (neutral fat) in

Fig. 1. Fat constituents of liver. Chemical analysis.
Fig. 2. Infra-red absorption spectrophotogramm.

Fig. 3. Schematic presentation of area ratio of fatty acids. Gas chromatography.
frozen section and lipase was noticed. Namely, when there was no noticeable fat infiltration, abundant lipase was disclosed in corresponding liver and no lipase activity was demonstrated in fat infiltrated areas. The converse held good (Figures 4, 5, 6 and 7).

Comparative study between fatty acid content determined by means of

![Fig. 4 Remarkable fat infiltration in central area of liver acini is noticed. Yellow rice fed mouse. Sudan III stain, ×42.](image1)

![Fig. 5 Lipase is more intensely demonstrated in peripheral area of acini than central region. Same mouse with Fig. 4. Lipase (Tween 20) stain, ×42.](image2)

![Fig. 6 Scanty fat infiltration is noticeable in pseudolobuli. Atrophic cirrhosis of liver. Sudan III stain, ×40.](image3)

![Fig. 7 Abundant lipase is noticed almost throughout in pseudolobuli. Same case with Fig. 6. Lipase (Tween 20) stain, ×40.](image4)

Table 1. Relation Between Fatty Acid Amounts and Lipase Activity

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Autopsy Diagnosis</th>
<th>Gastric Cancer</th>
<th>Hepatoma</th>
<th>Cirrhosis of liver</th>
<th>Fatty liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid</td>
<td>mg/100g*</td>
<td>Lipase**</td>
<td>mg/100g*</td>
<td>Lipase**</td>
<td>mg/100g*</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>0</td>
<td>#</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>1105</td>
<td>+</td>
<td>379</td>
<td>+</td>
<td>731</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>512</td>
<td>+</td>
<td>252</td>
<td>+</td>
<td>309</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>1000</td>
<td>±</td>
<td>283</td>
<td>#</td>
<td>495</td>
</tr>
<tr>
<td>Linolenic Acid</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

* Fatty acid amount determined by means of gas chromatography.
** Lipase indicated lipase acitvity using corresponding substrate.
- : negative, ± : weak positive, + : positive, # : strongly positive.
gas chromatography and lipase activity disclosed that when a certain fatty acid increased in amount, there was relatively proportionate decrease of corresponding lipase activity (Table 1). Similar relation between the total fat content and lipase activity was also noticed.

Conclusion

Histochemical demonstration of lipase and analytical studies of liver fat were carried out. From the above-described findings, it could be concluded that histochemically demonstrable lipase in liver will precisely reflects the amount of liver fat or fatty acids as well as the fat metabolism in liver.

Reference


Discussion

Dr. Mizutani:
1. Histochemical methods of lipase, as you know, use tweens as substrate, not found in animal tissues. Since Gomori, there are some discussions about the specificity of these reactions, especially in regard to the relationship between lipase and esterase, and between hepatic and pancreatic lipases. We must pay such as considerations in any studies of lipase and esterase.
2. And secondly we have experienced the seasonal change of activity that rat tissues showed the strong reactions for the tween method in winter and weakened in summer.

Dr. Okudaira: Thank you very much for your comments. On the second subject we have been performed a extensive investigation for the standardization of lipase staining, but we had no such experience.

Histochemical Studies On Lipid Metabolism.

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The lack of suitable histochemical methods for the study of lipid metabolism of tissues has delayed solutions of many important problems. Although various methods, making use of physico-chemical differences in the nature of the lipids, have been proposed for the fixation of various lipids contained in tissues, there