The mechanism of the release of hepatic enzymes in various liver diseases. 1. Alterations in cytoplasmic and mitochondrial enzyme activities in serum.

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Abstract

Serum glutamic oxaloacetic transaminase (GOT), mitochondrial GOT (GOTm), glutamic-pyruvic transaminase (GPT) and glutamate dehydrogenase activities were determined in 43 healthy controls and in 280 cases of liver diseases. A simplified column chromatographic method coupled with UV assay was employed for separation of GOTm. The activity was measured by following decrease in abosrbance of NADH at 340 nm. The lowest activity of GOTm determined with a coefficient of variation below 10% was 6 mIU/ml. High GOTm activities were found in acute hepatitis (acute stage), subacute hepatitis and primary biliary cirrhosis and were generally associated with high total GOT (GOTt) activities. The activity ratio of GOTm/GOTt varied depending on the stage and severity of liver diseases. The GOTm/GOTt ratio was decreased in acute, fulminant and subacute hepatitides. No significant reduction in the ratio was found in bile duct obstruction, alcoholic liver injury or metastatic liver cancer. Although relatively high GOTm/GOTt ratios were found in some patients with severe hepatic injury, they had no definite association with poor prognosis. These results indicate that the marked elevation in GOTt over GPT in advanced chronic hepatitis, liver cirrhosis and primary hepatoma was mainly due to preferential leakage of cytoplasmic GOT (GOTs).

KEYWORDS: glutamic-oxaloacetic transaminase, mitochondrial glutamic-oxaloacetic transaminanse, glutamic-pyruvic transaminase, liver diseases

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THE MECHANISM OF THE RELEASE OF HEPATIC ENZYMES IN VARIOUS LIVER DISEASES
1. ALTERATIONS IN CYTOPLASMIC AND MITOCHONDRIAL ENZYME ACTIVITIES IN SERUM

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Abstract. Serum glutamic-oxaloacetic transaminase (GOT), mitochondrial GOT (GOTm), glutamic-pyruvic transaminase (GPT) and glutamate dehydrogenase activities were determined in 43 healthy controls and in 280 cases of liver diseases. A simplified column chromatographic method coupled with UV assay was employed for separation of GOTm. The activity was measured by following decrease in absorbance of NADH at 340 nm. The lowest activity of GOTm determined with a coefficient of variation below 10% was 6 mIU/ml. High GOTm activities were found in acute hepatitis (acute stage), subacute hepatitis and primary biliary cirrhosis and were generally associated with high total GOT (GOTt) activities. The activity ratio of GOTm/GOTt varied depending on the stage and severity of liver diseases. The GOTm/GOTt ratio was decreased in acute, fulminant and subacute hepatitides. No significant reduction in the ratio was found in bile duct obstruction, alcoholic liver injury or metastatic liver cancer. Although relatively high GOTm/GOTt ratios were found in some patients with severe hepatic injury, they had no definite association with poor prognosis. These results indicate that the marked elevation in GOTt over GPT in advanced chronic hepatitis, liver cirrhosis and primary hepatoma was mainly due to preferential leakage of cytoplasmic GOT (GOTs).

Key words : glutamic-oxaloacetic transaminase, mitochondrial glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, liver diseases

Various enzymes originating from tissues are found in the serum, and the changes in their activities have been correlated with disease process. Glutamic-oxaloacetic transminase (GOT; L-aspartate : 2-oxoglutarate aminotransferase, EC 2. 6. 1. 1) and glutamic-pyruvic transaminase (GPT; L-alanine : 2-oxoglutarate aminotransferase, EC 2. 6. 1. 2) are the most widely used in monitoring liver diseases. However, the clinical significance of increased GOT and GPT activities in serum and of the altered activity ratio of GOT to GPT is still poorly understood.

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Since two isozymes of GOT are known to exist (1-3), one in cytoplasm (GOTs) and another in mitochondria (GOTm), the contribution of each to the total GOT (GOTt) activity might differ depending on the type of parenchymal liver injury involved. The appearance of GOTm in the serum of patients with viral hepatitis and liver cirrhosis has been evaluated clinically by Kawaguchi *et al.* (4). The isolation technique they used was complicated and limited in sensitivity. Thus, clinical application of the technique is difficult even though estimation of GOTm is considered of potential clinical use.

In the present study, a small-scale column method (5) developed as a simplified technique for the clinical laboratory was used to determine the contribution of GOTm to the elevation of GOTt activity in various pathological conditions of the liver. Although Schmidt *et al.* (6) measured the level of GOTm in serum of patients with liver diseases, the results were compared only with glutamate dehydrogenase (GLD; L-glutamate : NAD oxidoreductase (deaminating), EC 1. 4. 1. 2) without reference to GOTm/GOTt ratios. Generally, the appearance of GOTm in serum has been considered *a priori* to reflect severe hepatocyte damage. GOTm and GOTs might leak through due to altered membrane permeability (7-9) or be released into the blood stream as a result of liver cell destruction (6, 10, 11), or either of them might appear preferentially in the plasma by one of these two mechanisms. Because of the distinct intracellular localization of these isozymes, it is reasonable to assume that the ratio of GOTs to GOTm varies depending on the type of hepatocyte injury involved. From the same point of view, the activities of other enzymes with well-defined cytoplasmic or mitochondrial localization (such as GPT, mostly present in the cytoplasm, and GLD, exclusively confined to the mitochondria) were determined in addition to GOTs and GOTm. This paper presents the results obtained in acute and chronic hepatitides, liver cirrhosis and primary hepatoma.

MATERIALS AND METHODS

Sera were collected before breakfast from 43 healthy controls (male 22 and female 21) and 280 cases of various liver diseases. Diagnoses of them were made by liver function tests and peritoneoscopic and histological examinations of the liver. The sera were kept at 4°C and analyzed for the following enzymes within three days without loss of activity.

Serum GOT, GPT, GOTm and GLD activities were determined by following the rate of NADH oxidation at 340 nm with a Gilford recording spectrophotometer, Model 240, with the cuvette chamber set at 30°C. GOT and GPT assays were made with reaction mixtures obtained from Boehringer Mannheim Co., Mannheim, Germany according to the method of Karmen *et al.* (12) and GLD activities were analyzed by the method of Olson *et al.* (13). Usually, 100 μl serum was used for GOT and GPT, 200 μl serum for GLD and 300 μl eluate from 50 μl

applied serum for GOTm in a final volume of 1 ml of reaction mixture, and the reagents were warmed up at 30°C beforehand. Small activities found for the blank cuvette, which received water in place of serum, were subtracted from the activities in the complete system. Enzyme activities were expressed as mili international units per ml of serum.

GOTm was separated by a small-scale column method (Nippon Chemiphar Co. Ltd., Tokyo) at room temperature and activities were measured within 1 h. GOTs activities were calculated by subtracting GOTm activities from the GOTt.

Cellogel (Chemetron, Milano, Italy) electrophoresis was done with barbital buffer ($\mu = 0.05$) at 250 V for 1 h (1). After electrophoresis, the Cellogel membrane was placed on the filter paper soaked with substrate and enzyme solutions for GOT, incubated for 20 min at 37°C and the oxidised NADH band visualized by a UV lamp (long wave 3650 Å, Manaslu Co., Tokyo).

Determination of GOTm activity with specific antibody (antibody method) (14, 15) was performed with a GOT isozyme test kit (Eiken Chemical Co., Tokyo) by adsorbing GOTs with goat anti-GOTs blood for 5 min at room temperature, then analyzing the supernatant GOTm fraction after centrifugation at 3000 r.p.m. for 3 min.

RESULTS

The coefficients of variation (CV) in assay of GOTt in the lower range of activities are given in Fig. 1, indicating that GOTt activities above 2 mIU/ml
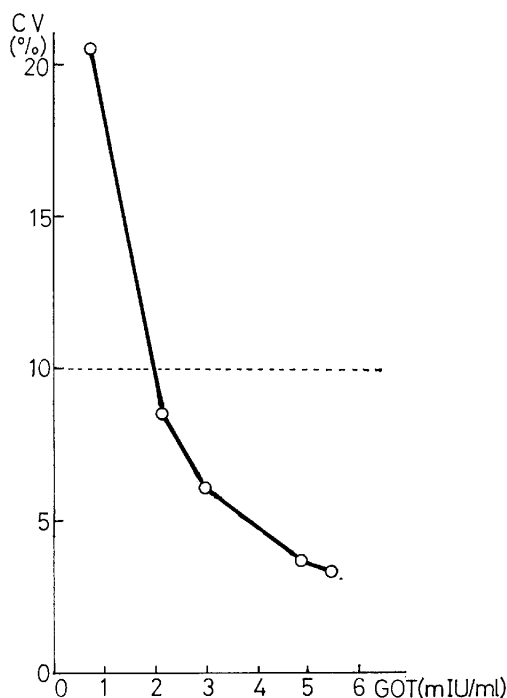

Fig. 1. CV's with varying GOTt activities. The number of determinations was 8.

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could be determined with CV's less than 10%. On the other hand, the CV's
for GOTm assay were greater than those for GOTt, and more than 6 mIU/ml
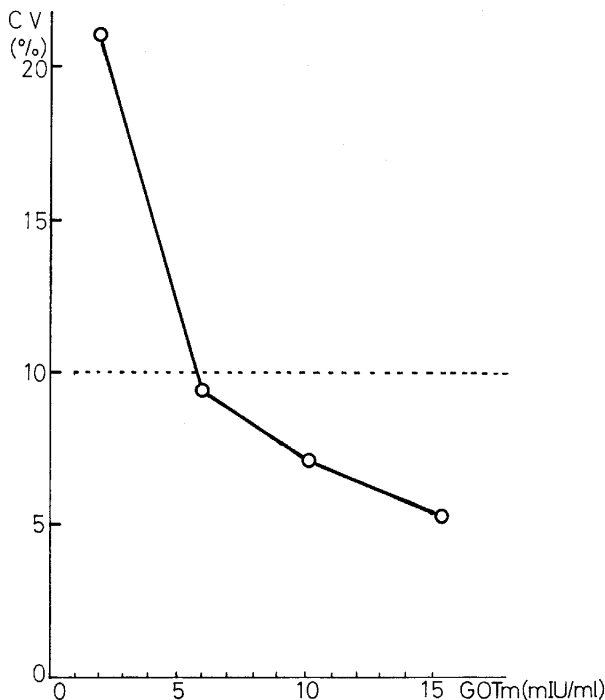of activity was required to minimize the CV below 10% (Fig. 2). Since the

Fig. 2. CV's with varying GOTm activities. The number of determinations was 8.

correction for volume of each eluate was made on GOTm assay, the larger CV
for GOTm than for GOTt at the same enzyme activities appeared to be caused
by the additional variation in the recovery of GOTm activity with the small-
scale column chromatography. The variation in recovery of GOTm activity
was determined and the results are shown in Fig. 3. The mean recovery with
serum GOTm activities above 6 mIU/ml was 88.2% with a CV of 6.8%.
GOTm activities as high as 1415 mIU/ml could be determined within assay
errors covered by the range shown in the figure. Since the recoveries of GOTm
with activities above 6 mIU/ml were satisfactory, no correction was made for
the small loss of activity in this procedure. However, the recoveries of small
GOTm activities less than 6 mIU/ml increased considerably. Therefore, the
individual values of single determination and the ratios derived from them were
not taken into account for the small GOTm activities unless a sufficient number
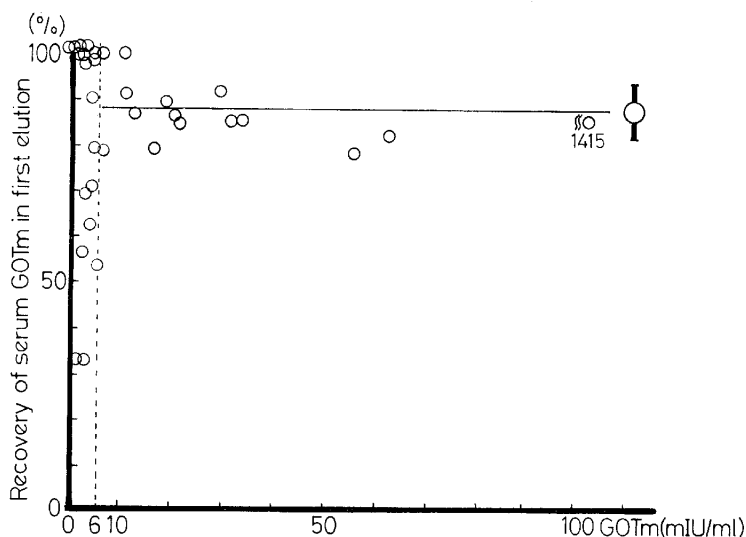of determinations was available. No GOTs activity was visualized on Cellogel

Fig. 3. Recoveries of serum GOTm in small-scale column chromatography. The activity of GOTm in the first eluation was divided by the total activity of GOTm in five successive elutions to give the recovery. GOTm activities after the third elution were negligible. The fraction eluted with 300 μl of 0.2 M phosphate (Na/Na) + 0.2 M NaCl buffer, pH 7.0 (5) gave no GOTm band with an intense GOTs band after electrophoresis on Cellogel membrane. Recovery of the first elution was 88.2 ± 6.0% (shown by a large open circle with vertical bar on the right) with a CV of 6.8% above 6 mIU/ml in GOTm activity as indicated by the horizontal line.

electrophoresis of a GOTm eluate with an activity of 218 mIU/ml, indicating no practical contamination of GOTs in the GOTm fraction (Fig. 4). The GOTm activity determined by the antibody method was 10 to 80% higher than that by the column method in all the cases studied with GOTt activities below 1000 mIU/ml. This was found to be due to contamination of the GOTm fraction with GOTs in the antibody method and not due to insufficient elution of GOTm in the chromatographic method as revealed by electrophoresis on Cellogel membrane (Fig. 5). The extent of contamination was independent of GOTt or GOTm activities.

When serum was kept at 4°C, no significant loss of GOTt, GOTm, GPT and GLD activities was observed for 7 days (Fig. 6). Inactivation of GOTm after elution from the column was negligible at room temperature up to 8h (Fig. 7).

The activities of GOTt, GOTm, GPT and GLD in the serum of healthy controls and of patients with liver diseases are summarized in Table 1. No significant difference in GOTm activity was found between male and female. The mean GOTm activity was elevated in acute hepatitis (acute stage with GOTt

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GOTt 4712 mIU/ml
GOTm 1415   "

Fig. 4. Cellogel electrophoresis of GOTm and GOTs fractions. GOTm fraction, the first eluate; GOTs fraction, the eluate with 0.2 M phosphate (Na/Na)+0.2 M NaCl buffer, pH 7.0, after the first elution. Serum from a case of acute hepatitis with GOTt activity of 4712 mIU/ml and GOTs activity of 1415 mIU/ml was employed.
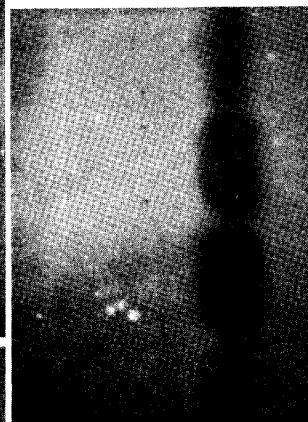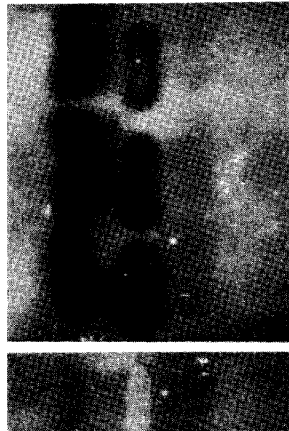
Fig. 5. Cellogel electrophoresis of GOTm fractions by antibody method and GOTs fractions by chromatograhpic methods. A, Antibody method; B, Chromatographic method. Four sera with different GOTt activities from patients with different GOTt activities from patients with acute hepatitis were used. Approximately 8 μl samples were applied. Dark areas at the origin in the electrophoretograms are due to non-specific coprecipitation of GOT.
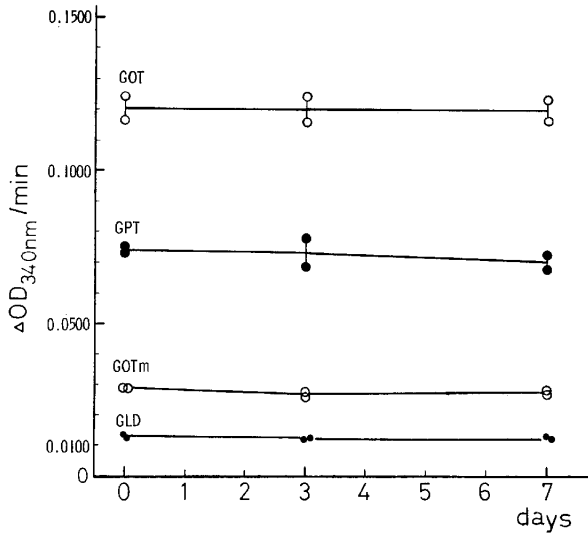
Fig. 6. Activities of GOTt, GOTm, GPT and GLD during the storage of serum at 4°C. Individual values of two assays and their means are shown.
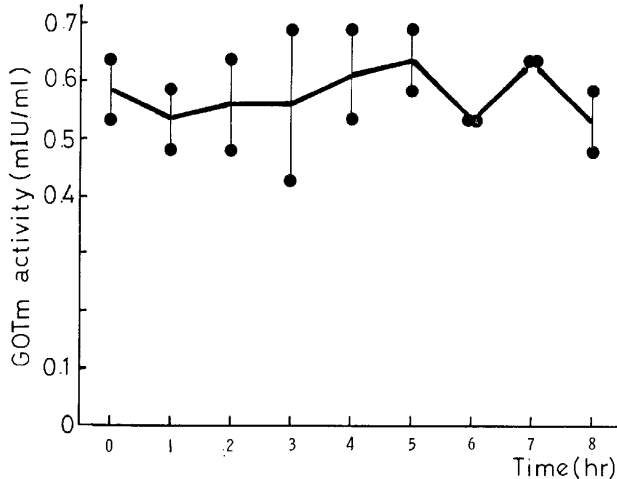

Fig. 7. GOTm activities in eluate kept at room temperature for different periods of time. Individual values of two assays and their means are shown.

activities above 150 mIU/ml) and moderately increased activity in subacute hepatitis, primary biliary cirrhosis, fulminant hepatitis and biliary obstruction. The increase in GOTm activity roughly paralleled the increase in GOTt, GPT and GLD activities. The distribution of GOTm activity varied widely among disease groups, particularly in acute hepatitis (acute stage), fulminant hepatitis,

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TABLE 1. Serum activities of GOTt, GOTm, GPT and GLD in healthy
controls and in patients with livtr diseases.

| Diagnosis	No. of cases	Activities (mIU/ml)			
		GOTt	GOTm	GPT	GLD
Healthy controls	43	8.6±3.1	0.7±0.4	5.5±2.8	0.2±0.1
Acute hepatitis, acute stage	23	645.7±736.7	70.4±236.7	575.4±283.9	13.6±28.5
Acute hepatitis, convalescence	23	53.7±40.3	2.6±1.6	78.3±68.6	1.5±3.6
Subacute hepatitis	6	369.5±230.8	7.5±6.8	296.5±175.7	3.2±2.8
Fulminant hepatitis	8	190.4±144.8	5.3±5.0	300.5±346.0	1.5±2.2
Chronic hepatitis	51	49.8±54.3	2.5±2.4	54.6±65.1	1.0±1.1
Liver cirrhosis	42	54.3±52.9	1.8±1.1	35.3±28.2	1.1±1.3
Hepatoma with liver cirrhosis	34	96.2±109.5	3.9±3.6	24.4±28.6	1.6±2.6
Malignant bile duct obstruction	11	59.5±46.5	5.3±6.4	39.6±58.2	3.4±5.1
Cholelithiasis	5	42.8±24.9	4.8±2.7	46.8±32.1	4.7±3.3
Intrahepatic cholestasis	10	43.7±32.6	3.6±5.0	41.7±41.5	1.1±2.0
Primary biliary cirrhosis	4	103.0±59.9	6.8±2.7	56.5±26.7	3.5±1.1
Cancer, liver metastasis (+)	15	39.3±28.7	5.0±4.1	16.1±19.3	0.9±1.2
Cancer, liver metastasis (−)	10	11.7±7.8	0.8±0.5	5.9±6.3	0.5±0.3
Alcoholic livnr injury	28	42.6±42.0	4.4±4.9	26.6±25.3	1.3±1.7
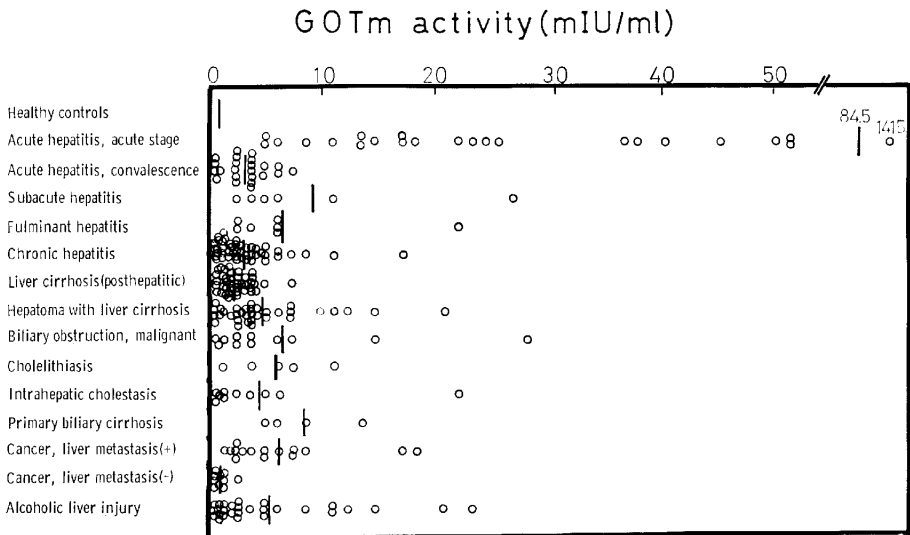
Fig. 8. GOTm activities in sera of healthy controls and of patients with liver diseases.
Cases with GOTt activities more than 150 mIU/ml in acute stage of acute hepatitis are in-
cluded. Individual values in healthy controls are not given because of limitation in spacc.
See Table 1 for distribution.

subacute hepatitis, malignant obstruction of bile duct, metastatic liver cancer and alcoholic liver injury (Fig. 8). The extent of the increase in GOTm activity in acute hepatitis (acute stage) appeared to depend on the severity of the hepatic injury as reflected by the rises in GOTt or GPT activities, because the rise in GOTm activity in this group correlated well with that of GOTt ($r = 0.952$, $P < 0.01$) or GPT ($r = 0.663$, $P < 0.001$).

The degree of contribution of the increase in GOTm to that of GOTt was relatively small. The percent activities of GOTm in GOTt in healthy controls and in patients with liver diseases are given in Fig. 9. The ratio varied widely in each group, ranging from 0 to 55.9%. Although this could be attributable
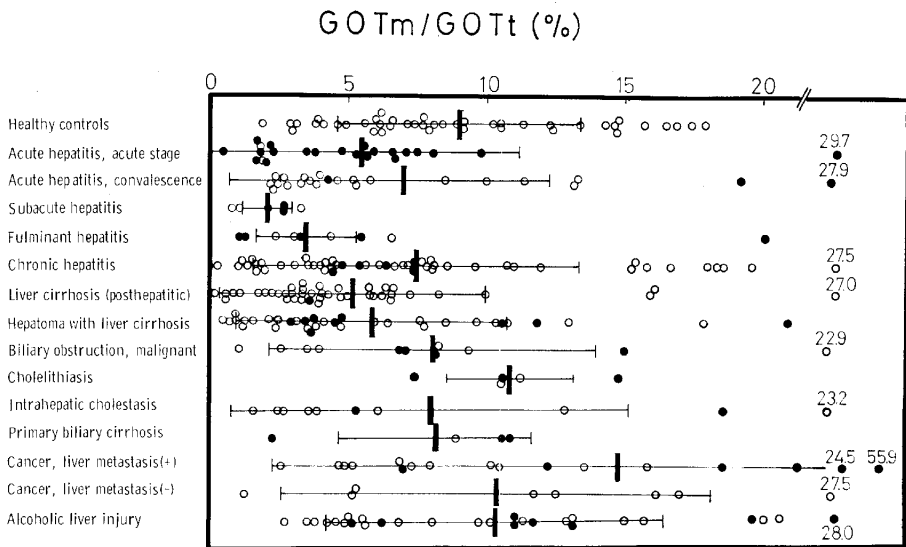
G O Tm / G O Tt (%)

Fig. 9. Activity ratios of GOTm/GOTt in healthy controls and in patients with liver diseases. ●, GOTm$\geq$6 mIU/ml and ○, GOTm$<$6 mIU/ml.

in part to the variation in determination of small GOTm activities, no apparent difference was found in the distribution between the cases with GOTm activities below and above 6 mIU/ml. The mean GOTm/GOTt ratio was 9% in healthy controls and significantly decreased ($p < 0.01$) in acute hepatitis (acute stage) even though GOTt activity was markedly increased. The ratio was also slightly lower in convalescent acute hepatitis than in healthy controls, although the GOTt activity was much lower. Lower ratios were also observed in subacute ($P < 0.001$) and fulminant ($P < 0.01$) hepatitides. In liver cirrhosis and primary hepatoma, where GOTt activity is predominantly increased over GPT activity, the GOTm/GOTt ratio was signficantly diminished ($P < 0.001$ for liver cirrhosis

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and P<0.01 for primary hepatoma), indicating that the preferential increase in serum GOTt over GPT was not due to increase in GOTm activity. CK (Creatine kinase) activity was not elevated in 16 cases with high GOTm activities including severe cases. In bile duct obstruction, alcoholic liver injury and carcinoma with liver metastasis, lower GOTm/GOTt ratios were not observed even if the increase in GOTt activity was relatively large compared with the rise in GPT activity.   Thus, the contribution of GOTm to the elevation of GOTt is considerably large in these liver diseases.

A similar tendency was found in GLD/GOT ratio in activity (Fig. 10), although the difference among diseases of the liver and biliary system was less
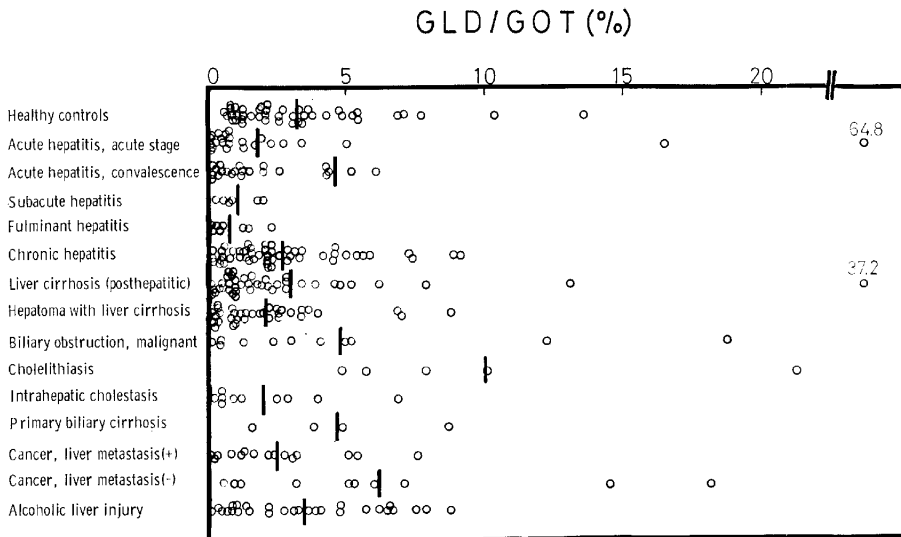
GLD / GOT (%)

Fig. 10. Activity ratio of GLD/GOT.

marked, because the alteration of GOTm was included in GOTt as a contributing factor. In this regard, the change in GOTm might parallel that of GLD, and this was confirmed by direct comparison of these activities, although some high ratios of GOTm/GLD were found in metastatic liver cancer (P<0.01) and acute hepatitis (acute stage) (P<0.02) and lower ratio in cholelithiasis (P< 0.05) as compared with the healthy controls (GOTm/GLD=4.2±3.0).

The GOTm/GOTt ratio tended to decrease as the GOTt activity became high in acute hepatitis, chronic hepatitis and liver cirrhosis (Fig. 11). In bile duct obstruction and alcoholic liver injury, no significant correlation was found between the GOTm/GOTt ratio and the extent of GOTt elevation. The findings that the GOTm/GOTt ratios were low in cases with high GOTt activities
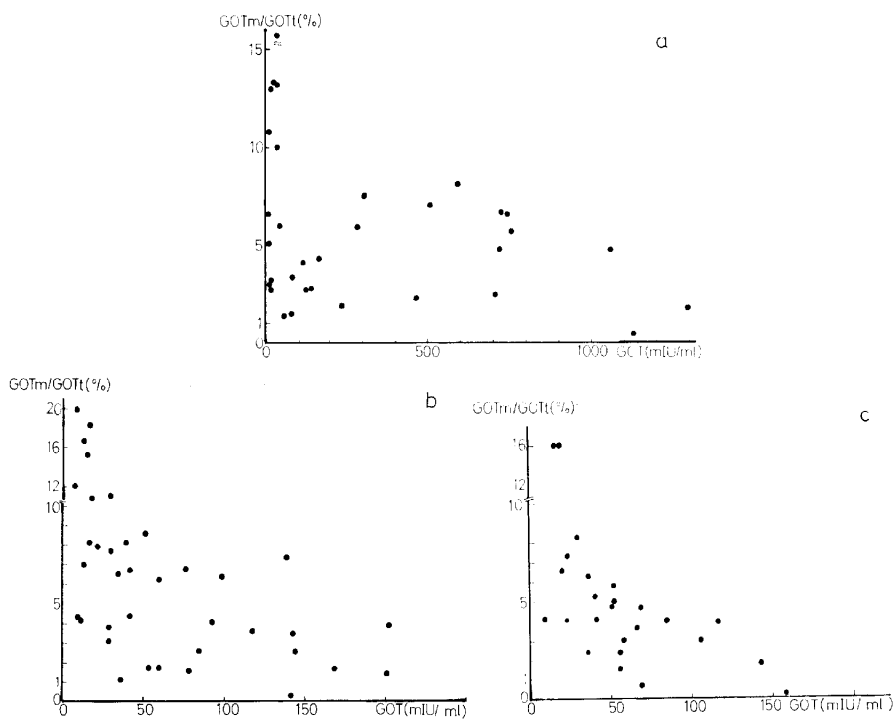
Fig. 11. GOTm/GOTt ratio with varying GOTt activities in the serum. a, acute hepatitis ($r = -0.453$), b, chronic hepatitis ($r = -0.512$) and c, liver cirrhosis ($r = -0.569$).

were also confirmed by following changes in the GOTm/GOTt ratio during the course of severe liver diseases. In acute hepatitis with moderate elevation of GOTt activities, the GOTm/GOTt ratio decreased as the GOTt activity reached a peak and then returned toward the normal range (Fig. 12 a and b). A similar clockwise rotation of the GOTm/GOTt vs. GOTt relation was observed in all of 5 cases with markedly elevated GOTt, although there was an initial increase in GOTm/GOTt ratio (Fig. 12 c). In terminal cases of liver cirrhosis with or without primary hepatoma (Fig. 13), there was a relative increase in the GOTm/GOTt ratio a few days before death. The GOTm/GOTt ratio increased in 3 fatal cases among 4 cases of fulminant hepatitis, the ratio reaching as high as 31.5%. In acute myocardial infarction (Fig. 14 a, b, c and d), where myocardial necrosis could be assessed by ECG and CPK analyses (16–18), the ratio of GOTm/GOTt and GOTt activity were followed in 5 cases. The contribution of GOTm in the peak of GOTt elevation following the attack was relatively small (less than 15%) in survived cases. In one unsustained case (Fig. 15 a), the initial ratio had already risen to 20%.
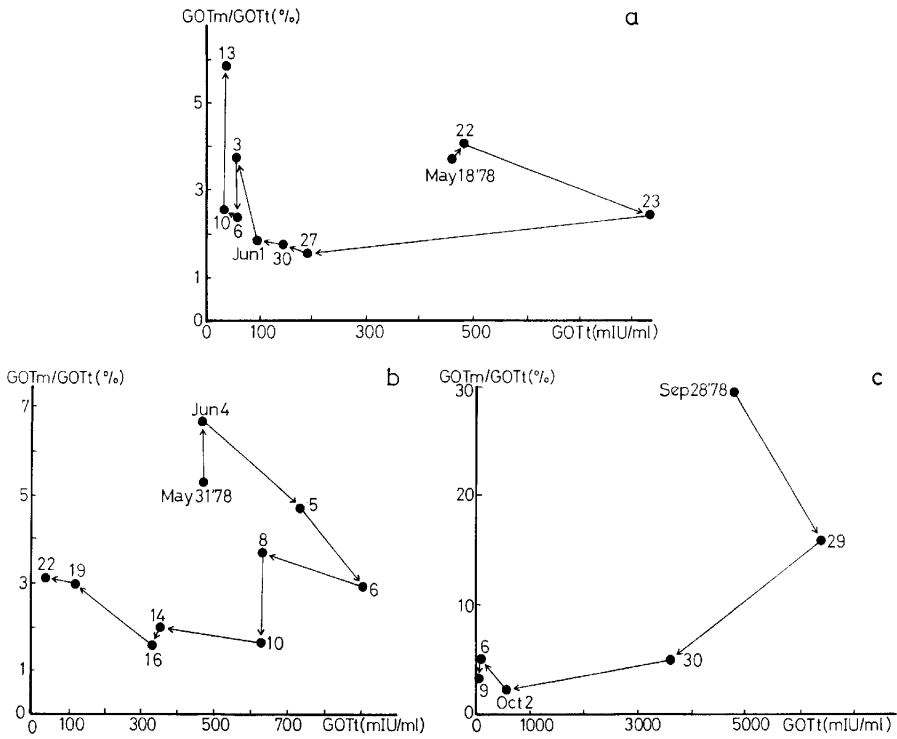
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Fig. 12. Follow up cases of acute hepatitis for the GOTm/GOTt ratio. a, case I. F. ; b, T. O. ; and c, K. T.
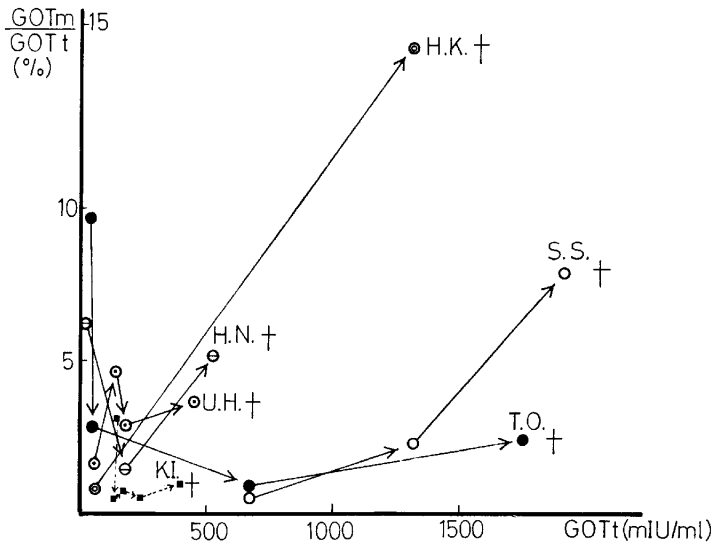

Fig. 13. Contribution of GOTm to the terminal rise in GOTt activity in primary hepatoma with liver cirrhosis. Individual cases are shown by different symbols connected by **arrows.**
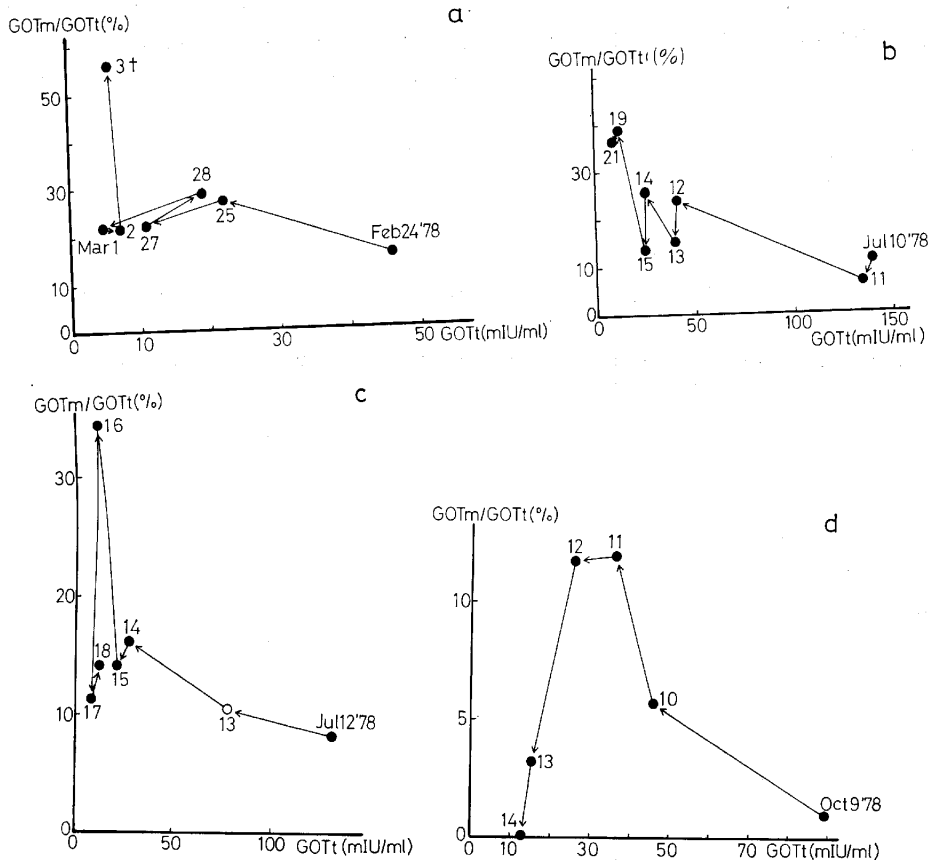
Fig. 14. Follow up of the GOTm/GOTt ratio in cases of myocardial infarction. a, F. M.; b, T. O.; c, H. F.; and d, Y. K.

DISCUSSION

The release of hepatic enzymes with different intracellular localization into the blood plasma was studied by Kawaguchi et al. (18) with varying liver injuries of experimental animals and clinical materials. Although relatively uniform elevation of enzymes in different cellular organelles, including GOTm, were found in serum in acute carbon tetrachloride intoxication of rats, only in cases of acute hepatitis with relatively high serum GOTt, was GOTm detected in serum with a maximum of 6% of GOTt activity. With the ordinary column chromatographic method, the number of cases analyzed for serum GOTm is limited because of the time-consuming procedure.

Since the development of a small-scale column chromatography or specific antibody technique for separation of GOTm from GOTt, these newly developed

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methods enabled us to determine small activities of GOTm with minute quantities of serum. However, the lowest activity of GOTm we could determine with CV below 10% was 6 mIU/ml even with the small-scale column method combined with a sensitive spectrophotometric assay. Thus, activities below this level were not considered as individual values, this applying to the healthy control activities.

The GOTm/GOTt ratios determined by the present method fell in the range from 2.9 to 17.9% with a mean ratio of 9.0. The ratios in most of the cases studied, however, agree well with those reported by Kawaguchi and others with a large-scale column method (5). On the other hand, the GOTm/GOTt ratios determined by the specific antibody method are high (14, 15, 20). In direct comparison of GOTm activities of identical sera determined by the two methods, insufficient absorption of GOTs by the antibody was found in the present study to be the cause of high GOTm activities even using the indicated GOTt activities beolw 1000 mIU/ml.

The relatively small activities of serum GOT and GPT determined by the spectrophotometric assay, as compared with those determined by other kits, appeared to result from the assay kit itself, which was obtained from Boehringer Mannheim Co., in addition to resulting from the subtraction of small blank activities due to endogenous GOT activity contained in the reaction mixture.

The GOTm/GOTt ratios of 4.6 to 13.4% found in healthy control sera were smaller than those of 56.7 to 67.9% obtained with whole human liver homogenates of minimum hepatic involvement but close to those of 12.5 to 36.3% for human liver supernatants (5, 21). The difference between the serum and liver ratios could be explained mainly by the shorter half life of GOTm, 0.5–1 h (22, 23) or 6 h (6) than GOTs, 5–8 h (22, 23) or 12 h (6) and by assumed preferential release of GOTs from hepatocytes. The only substantial evidence supporting the latter assumption is that GOTm is tightly bound to the mitochondria (24, 25).

One of the interesting results emerged from the present study is that the GOTm/GOTt ratio decreased in serum of patients with acute hepatitis, subacute hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis and primary hepatoma with liver cirrhosis, a group of liver diseases with parenchymal injury as a common hepatic lesion. Since the clearance of GOT appears to be affected little by hepatic injuries (26), the results suggest that preferential release of GOTs occurs from the injured hepatocyte. This is in accord with the altered permeability of plasma membrane of damaged liver cells as demonstrated by several lines of indirect evidence (7-9, 27). Relatively little liver cell necrosis in acute hepatitis despite marked increases in serum GOT and GPT activities (27–30) suggest that the altered membrane permeability of injured hepatocytes leading to preferential GOTs (and also GPT) release does not necessarily cause liver

cell necrosis.

High GOTm activities in serum are generally regarded as a poor prognostic sign of hepatic injury (4, 20, 25). However, GOTt activity was also markedly increased in cases with high GOTm activities in the present study, indicating that the severity of hepatic lesion can not be inferred solely from the rise in GOTm activity. For example, the acute hepatitis cases with high GOTm activities had a fair clinical course. Furthermore, in some cases of acute parenchymal liver disease, including fulminant hepatitis and subacute hepatitis, relatively high GOTm activities as compared with those of GOTt were found with no definite association with poor prognosis. The relatively small contribution of GOTm to the increased GOTt in acute myocardial infarction with probable myocardial necrosis was also comparable to that in parenchymal liver injury (18). Accordingly, it is mere conjecture to assess the degree of hepatocyte necrosis from the increased GOTm/GOTt ratio as is so from GOT or GPT elevation (31).

Further support for this view derives from the higher GOTm/GOTt ratios found in obstructive jaundice and alcoholic liver injury (32) than in other type of acute parenchymal liver injuries, most of which are of viral-induced type. Since liver cell necrosis is not a predominant feature of bile duct obstruction and the parenchymal damage of alcoholic hepatitis in the cases studied was no more than that of other acute hepatitides, there appeared to be a selective mitochondrial damage in the obstructive and alcoholic liver diseases. This agrees with the results of morphological studies (33, 34).

A complex nature of enzyme release from impaired organelles of liver cells was evidenced by different activity ratios of GLD/GOT in the cases studied. Although Hayashi *et al.* (34) indicated relatively high GLD activities in cirrhosis of the liver, a more characteristic change of GLD/GOT ratio, namely a marked elevation, was found in cholelithiasis and a prominent decrease in fulminant hepatitis. GLD is considered to be readily released from mitochondria, particularly under hypoxic conditions (35, 36). Such experimental results can not be directly applied to the present clinical cases. For example, GLD activity was elevated not only in liver cirrhosis but also in bile duct obstruction and cholestasis. Thus, different and specific mechanisms of mitochondrial enzyme leakage into the blood appear to exist. Ornithine transcarbamylase, a liver-specific and mitochondrial enzyme, seems to be released more readily (24, 37–39).

The minor contribution of GOTm to GOTt elevation, despite marked increase of GOT as compared with GPT in liver cirrhosis and primary hepatoma, confirmed results reported by Kawaguchi *et al.* (4). Increase in the GOTm/GOTt ratio in primary hepatoma was found only as a premortem rise in GOTt activity. Many factors seem to contribute to the increase in GOTt and GOTm

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activities in the terminal stage, although the effect of muscle damage is not likely
to be involved because of no significant rise in CK activity in the present study.
The contribution of muscle damage is also reported to be small in the cases of
alcoholic liver diseases with high GOTm activities (32). The marked elevation
of GOT over GPT in advanced chronic hepatitis and liver cirrhosis in the present
study was due mainly to leakage of GOTs. This reflects an increased GOT/GPT
ratio in the injured liver cells as will be discussed in a subsequent paper (21).

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