Acta Medica Okayama

Volume 16, *Issue* 3 1962 *Article* 1

JUNE 1962

Enzymatic studies of glucuronide formation in impaired liver. IV. Liver glucuronyl transferase activity and uridine diphosphate glucuronic acid content in viral hepatitis patients

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Abstract

The liver glucuronyl transferase (GT) activity and uridine diphosphate glucuronic acid (UDPGA) content in the patients with viral hepatitis were determined using 4-methyl umbelliferone (4-MU) as a glucuronide receptor. The results were as follows: 1. In acute viral hepatitis, the decrease in the GT activity was more remarkable in the later stage of the recovery. In chronic viral hepatitis, the GT activity was decreased in accordance with the increase in the degree of liver injury. Liver UDPGA content was significantly reduced only in postnecrotic cirrhosis. 2. The decrease or injury in the parenchymal liver cells caused a decrease in the liver GT activity. These quantitative reductions in the liver parenchyme were not the only factor for the alteration in the GT activity of the liver. The results of the present study suggested an involvement of a qualitative change in the liver GT activity in human liver injuries, especially in the early stage of acute viral hepatitis; namely, there might be even an activation of the liver GT other than the reduction resulting from the decrease in the liver parenchyme. 3. The decrease in the liver GT activity correlated significantly with the decrease in the salicylamide glucuronide formation in vivo, while the alteration in the liver UDPGA content failed to correlate with that in the glucuronide formation in vivo. It was suggested that the velocity of in vivo UDPGA production rather than the UDPGA content of the liver was as important a rate-limiting factor for the glucuronide formation in vivo as the liver GT activity.

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*PMID: 13984680 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **16**, 115—128 (1962)

ENZYMATIC STUDIES OF GLUCURONIDE FORMATION IN IMPAIRED LIVER

IV. LIVER GLUCURONYL TRANSFERASE ACTIVITY AND URIDINE DIPHOSPHATE GLUCURONIC ACID CONTENT IN VIRAL HEPATITIS PATIENTS

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Received for publication, March 28, 1962

It has been well recognized that glucuronide formation takes place primarily in the parenchymal liver cells. And it seems probable that the glucuronide formatiom might be altered more or less by hepatocellular impairments. Although this alteration in human subject has been widely investigated, the results were inconsistent. NASARIJANZ[1] demonstrated a reduced menthol glucuronide synthesis in various affections of the liver parenchyme. ENGLERT and others[2], PETERSON and others[3], and STREETEN[4] commented that the conjugation of adrenocortical steroids with glucuronic acid in liver injuries was not impaired, while COHN and BONDY[5] reported that glucuronide conjugation was a rate-limiting step for the steroid metabolism in Laennec's cirrhosis. KUNIN and others[6] indicated a decrease in the glucuronide formation of chloramphenicol in hepatocellular disease. BARNIVILLE and MISK[7] reported a reduced conjugation of salicylamide with glucuronic acid in advanced portal cirrhosis. SNAPPER and SALTZMAN[8,9] observed a urinary excretion of benzoyl glucuronate after administration of benzoic acid in the patients with liver disorders, although they also indicated a retarded excretion of the glucuronate in a more severe type of liver disturbance. SHARNOFF and others[10], by using the same experimental method as that of SNAPPER and SALTMAN, further confirmed that the benzoyl glucuronate excretion test was positive in all cases of acute viral hepatitis, but not in all cases of liver cirrhosis. MAROGG and WEGMANN[11] observed that the ratio of serum glucuronic acid to serum bilirubin in the patient with hepatitis was lower than that in the patient with obstructive jaundice. On the basis of this observation, they suggested an impaired glucuronide formation in liver damage. MONBOE[12,13] demonstrated a disappearance or a decrease in the glucuronic acid portion of the so-called ester-type direct-reacting bilirubin in jaundiced urine in the patients with severe liver damage. BILLING[14] suggested a metabolic insufficiency in bilirubin conjugation in hepatitis, especially in its

chronic cases, from the evidence that bilirubin diglucuronide in the blood was decreased with the increase in the monoglucuronide in these cases. It was also noticed by SCHACHTER[15] that high levels of plasma bilirubin monoglucuronide were suggestive of hepatic parenchymal disease.

It is generally accepted that glucuronide formation involves a microsomal enzyme, glucuronyl transferase (GT), and uridine diphosphate glucuronic acid (UDPGA)[16,17,18,19]. However, no convincing data of enzymatic studies of glucuronide formation in the human liver affected by viral hepatitis were available[20]. In view of these conflicting results in the glucuronide formation in human liver injuries and of no satisfactory reports of enzymatic studies thereof, the GT activity and UDPGA content of the human liver tissues obtained by needle biopsy from the patients with viral hepatitis were measured in the present study in order to clarify the glucuronide formation in impaired human liver.

MATERIALS AND METHODS

Five normal subjects and thirty-seven patients with viral hepatitis hospitalized to Okayama University Hospital were selected. These patients included 8 with acute viral hepatitis, 5 with acute exacerbation of chronic viral hepatitis, 17 with chronic viral hepatitis, and 7 with postnecrotic cirrhosis. All these clinical diagnoses were confirmed by peritoneoscopy, histological studies, and liver function tests. The controls were all normal in those examinations and had neither the evidence of other diseases nor the history of hepatitis.

Liver tissues were obtained by percutaneous needle biopsy with the Vim-Silverman needle of a caliber 1.5 mm. under direct vision on peritoneoscopy. A portion of biopsy specimen of the liver was submitted to enzymatic and chemical assays and also to histological examinations. Immediately after liver biopsy, a portion of it, weighing 25 to 40 mg., was placed in an ice-cold small glass vessel and brought to the experimental room. The liver tissue was accurately and quickly weighed, actually within 30 seconds, by a torsion balance, and it was transferred into a well-iced 5 ml. teflon homogenizer. To this tissue, ice-cold, alkaline, isotonic potassium chloride[21] was added as a suspending medium to yield homogenate in a concentration of 2.5 per cent, and then homogenaization was performed in the ice water at a speed of 600 r. p. m. for ten to thirty seconds, depending on the amount of the tissue to be homogenized[22]. The GT activity and UDPGA content of the liver homogenate thus prepared were determined according to a modification[22] of the method of ARIAS[23] using 4-methyl umbelliferone (4-MU) as a glucuronide receptor. Each of two 0.4 ml. aliquots of the homogenate was used respectively for the assay of GT and UDPGA. Other 0.1 ml. aliquot of the homogenate was assayed for β-glucuronidase using p-nitrophenyl glucuronide as a substrate for the enzyme[24]. The incubations had to be started not more than

five minutes after removal of the tissue. Moreover, when the other 0.1 ml. aliquot of the homogenate was available, the total nitrogen of the homogenate was measured by the Kjeldahl method. On the serum drawn from the patient before the peritoneoscopic examination, measurements were further made of total and direct-reacting bilirubin[25] and enzyme activities of glutamic oxaloacetic transaminase (GO-T)[26], glutamic pyruvic transaminase (GP-T)[27], and β-glucuronidase[24]. The results of other liver function tests performed within one week before or after the examination were taken into account as a reference. These tests included zinc turbidity[28], thymol turbidity[29,30], bromsulphalein retention[31,32], and other enzyme activities, serum alkaline phosphatase[33,34] and serum choline esterase[35]. Twenty-one of these patients were submitted to a salicylamide glucuronide excretion test[36] three days after the examination.

RESULTS

The liver GT activities[1] of 5 normal controls were within the range of 12 to 15 mμ moles, and the mean of these activities was 13.52 mμ moles. The liver GT activities and UDPGA contents[2] in several stages of viral hepatitis are illustrated in Fig. 1. In acute viral hepatitis the liver GT activity was slightly decreased compared with that in the normal. This decrease in the liver GT activity was more remarkable in the later stage of the recovery when signs of jaundice disappeared from the patient and the results of the liver function tests were almost normal. On the other hand, the decrease in the liver GT activity was less remarkable when jaundice was still evident and the liver function of the patient was still considerably impaired. In exacerbated chronic viral hepatitis the GT activity diminished significantly in all of the cases, while in chronic viral hepatitis the diminution was significant in one half of the cases and in another half the activity remained within the normal range. The remarkable reduction in the GT activity was indicated in postnecrotic cirrhosis. The liver UDPGA contents in normal controls were within the range of 3 to 5 mμ moles, although considerable variations were observed in individual cases. The mean of liver UDPGA contents in 5 normal subjects was 3.89 mμ moles. The reduction in UDPGA content was marked only in postnecrotic cirrhosis. The GT activity in viral hepatitis patients with chronic colitis appeared to be rather higher than that in those without colitis, while the GT activity in viral hepatitis patients with cholecystopathy was rather lower than that in those without cholecystopathy. The alterations in the liver GT activity expressed on total nitrogen

1) Liver GT activity was expressed as mμ moles of 4-MU glucuronide formed per 10 mg. wet liver weight per 10 minutes.
2) Liver UDPGA content was expressed as mμ moles of 4-MU glucuronide formed per 10 mg. wet liver weight.

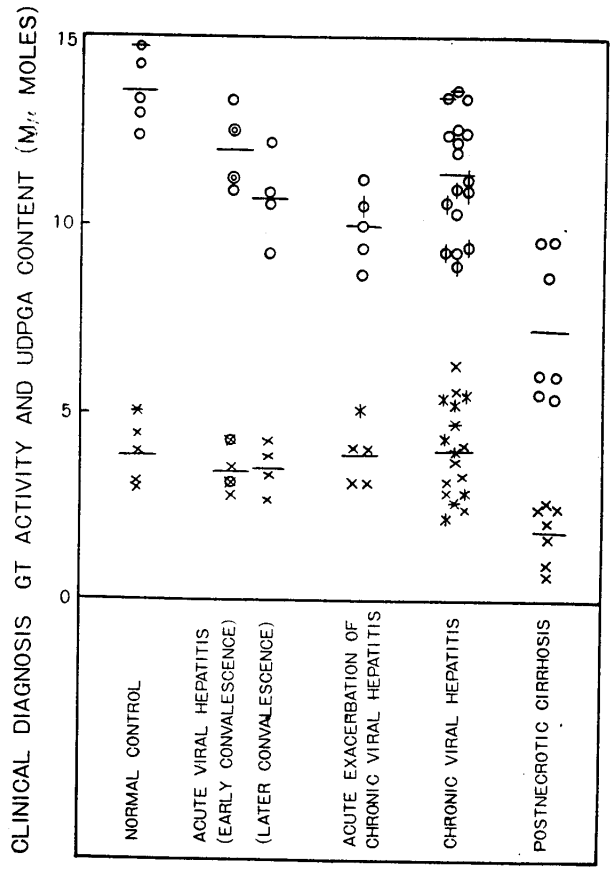

Fig. 1. GT activity and UDPGA content of liver tissue and the clinical courses in the patients with viral hepatitis. ○, GT activity; ×, UDPGA content; —, mean value. (◎, ⊠, case of cholangiolitic type; ⊖, ⊹, case with chronic colitis; ⊕, ⊹, case with cholecystopathy.)

basis were similar to those in the liver GT activity expressed on wet weight basis.

No significant correlations were obtained between the liver β-glucuronidase activity and the liver GT activity or UDPGA content.

The GT activity and UDPGA content of liver tissue in relation to the histological changes of the liver tissue are indicated in Fig. 2. In acute viral hepatitis, GT activity of the liver tissue with histological signs of the recovery was decreased significantly. In chronic viral hepatitis, the decrease in the liver GT activity was significant in the type I, while it was not considerable in the type II_B. The GT activities in the type II_A indicated that there was no apparent relationship between the inflammation in portal region and the alteration in the GT activity of the liver tissue. The decrease in the GT activity and UDPGA

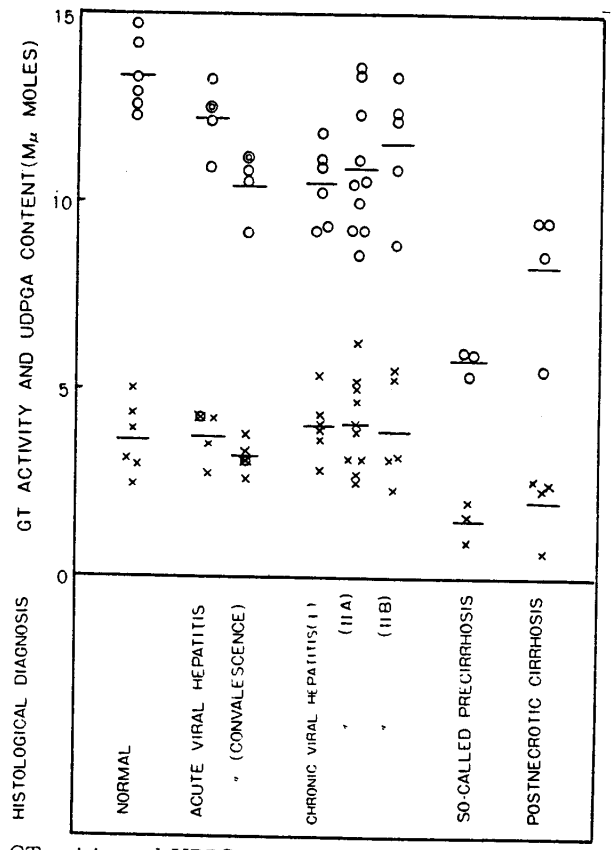

Fig. 2. GT activity and UDPGA content of liver tissue and the histological findings of the liver in the patients with viral hepatitis. ○, GT activity; ×, UDPGA content; —, mean value. (◎, ⊠, case of cholangiolitic type.) I, hepatocellular degeneration and necrosis without any significant changes in periportal and portal regions. II_A, persistent periportal cellular infiltrations with retention of normal lobular architecture. II_B, residual portal scarring with retention qf normal lobular architecture.

content of liver tissue was more profound in the precirrhosis characterized by the parenchymal liver-cell diminution resulting from massive necrosis and connective tissue proliferation.

As shown in Fig. 3, the GT activity of liver tissue appeared to correlate with the degree of necrosis or hydropic swelling of parenchymal liver cells. The GT activity of the liver tissue without any signs of liver-cell necrosis remained within the normal range. The GT activities of the liver tissues which demonstrated histologically so-called 'Spätknötchen' (Büchner) or spotty necrosis were slightly reduced as a whole, although some of them remained within the normal range. In the liver tissue with massive necrosis, the decrease in the GT activity

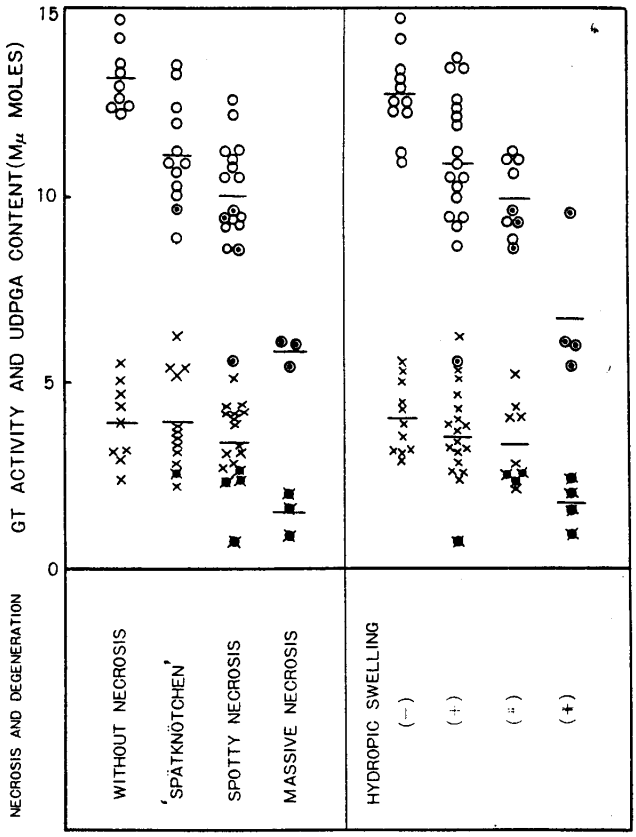

Fig. 3. GT activity and UDPGA content of liver tissue and necrosis or hydropic swelling of the liver cells in the patients with viral hepatitis. ○, GT activity; ×, UDPGA content; —, mean value. (⊙, ⊠, case with the proliferation of the connective tissue histologically more than 20 per cent of the liver tissue.) 'Spätknötchen', posthepatitis rest nodule.

was profound. Although there was no direct proportional relationship between the decrease in the GT activity of liver tissue and the decrease in liver parenchyme resulting from the increase in connective tissue, the GT activity of the liver tissue of which connective tissue was histologically more than twenty per cent was relatively lower than that of the tissue with a similar liver-cell necrosis and without the connective tissue proliferation. The decrease in the liver GT activity correlated slightly with the degree of hydropic swelling of liver cells, while the decrease failed to correlate with fatty or acidophilic degeneration of liver cells. The UDPGA content of liver tissue varied from case to case even in the subjects with normal histological findings of liver, and the relationship between the alteration in the UDPGA content and the histological changes of

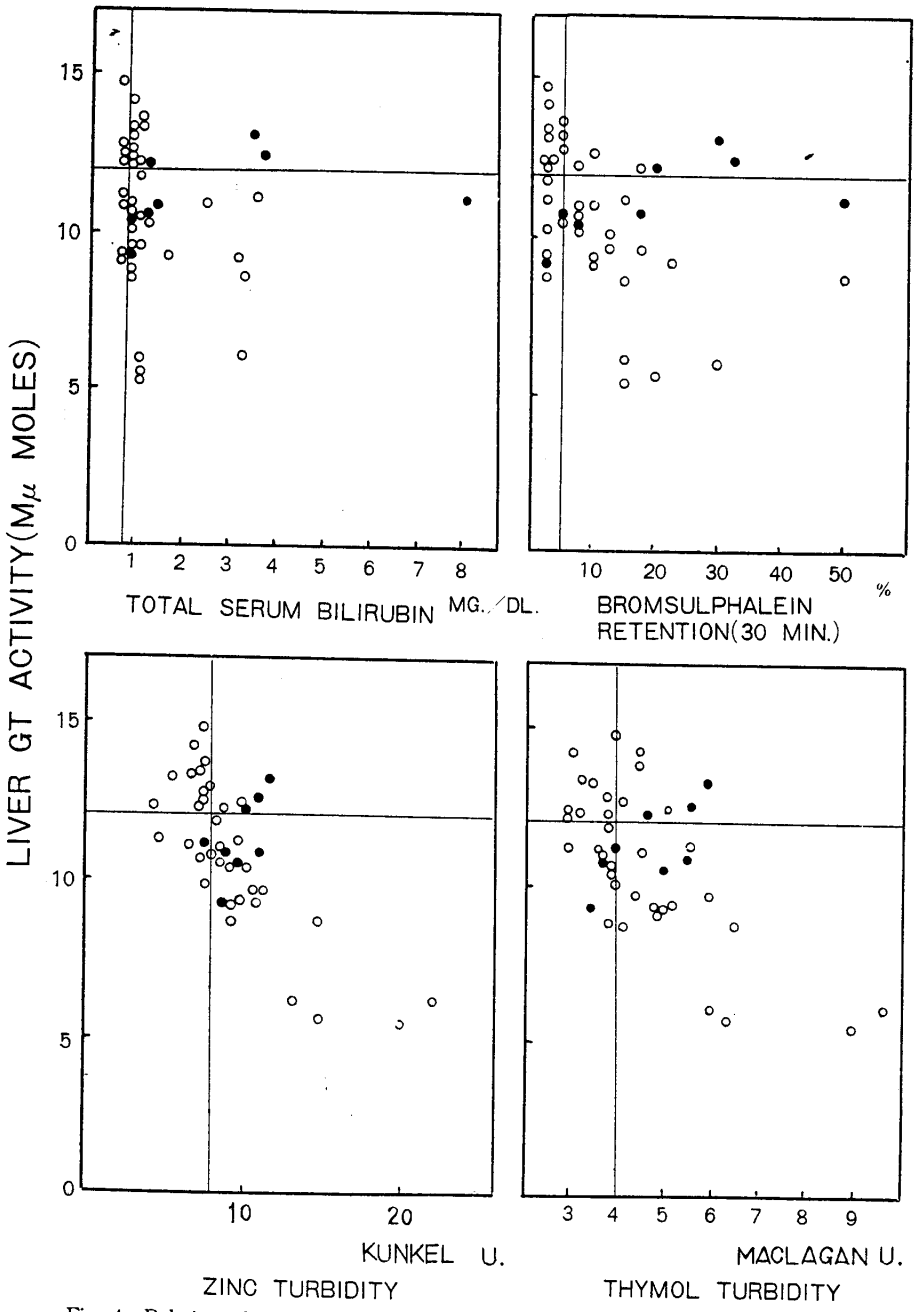

Fig. 4. Relation of liver GT activity to serum total bilirubin, bromsulphalein retention, zinc turbidity, and thymol turbidity in the patients with viral hepatitis. ●, liver GT activity in acute case; ○, liver GT activity in other case of viral hepatitis.

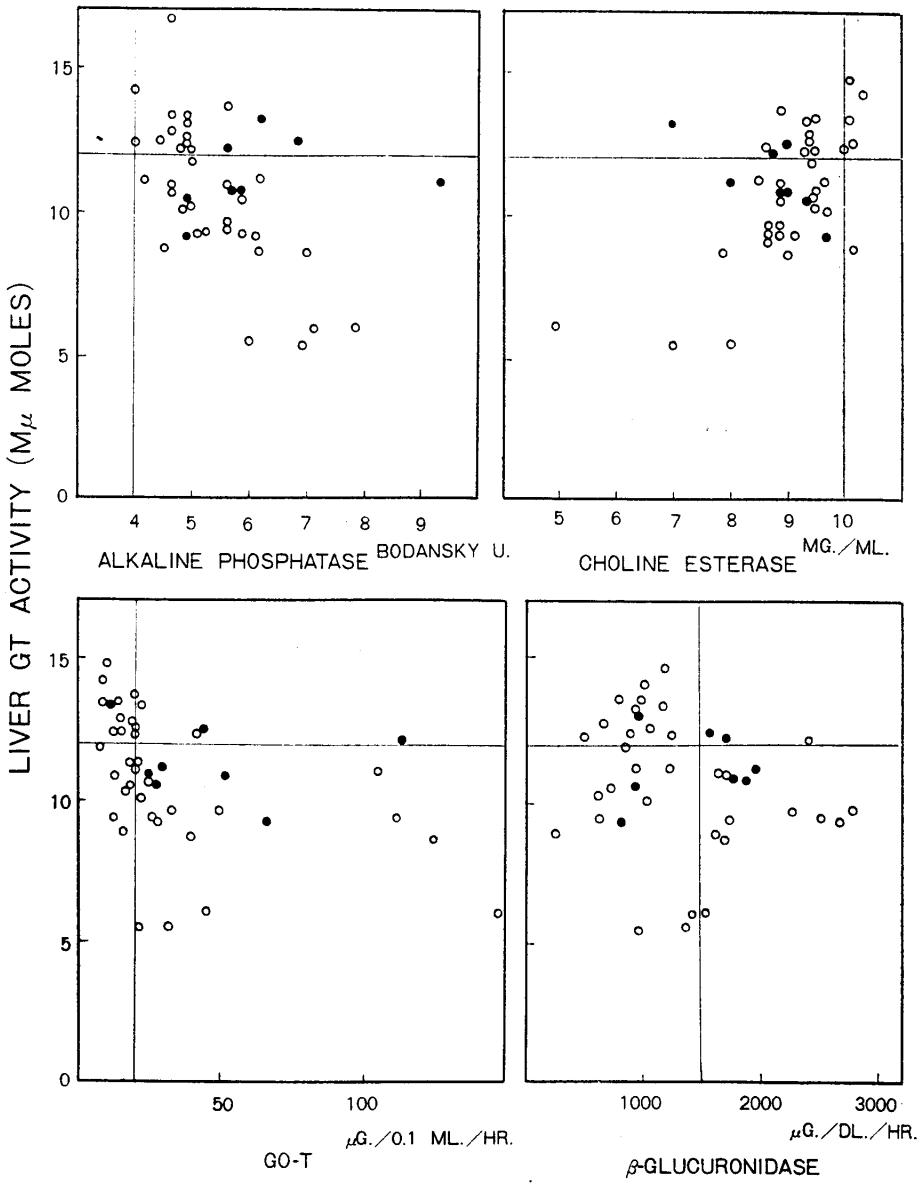

Fig. 5. Relation of liver GT activity to serum enzyme levels in the patients with viral hepatitis. ●, liver GT activity in acute case; ○, liver GT activity in other case of viral hepatitis.

the liver was not apparently indicated.

Alterations in the liver GT activity were studied in the light of serum biliru-

bin level, serum enzyme activities, and other liver functions, and the results are summarized in Figs. 4 and 5. The relationship between the liver GT activity and serum total bilirublin was not apparent as a whole. In acute viral hepatitis, however, the decrease in the GT activity was rather remarkable in the case with lower serum total bilirubin than in the case with higher serum total bilirubin. The similar relationship between the liver GT activity and serum direct-reacting bilirubin was also observed. In acute viral hepatitis, it was also noticed that the GT activity was diminished more markedly in accordance with the improvement in the liver function as revealed by bromsulphalein retention, zinc turbidity, thymol turbidity, serum alkaline phosphatase, and serum choline esterase. In other casse of viral hepatitis, on the contrary, the GT activity was reduced in accordance with the increase in the degree of impairment in the liver

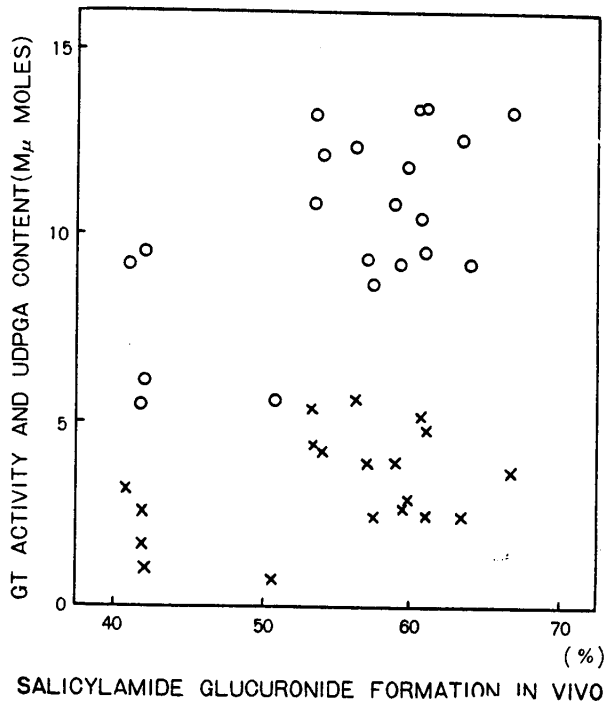

Fig. 6. GT activity and UDPGA content of liver tissue in relation to the capacity of salicylamide glucuronide formation *in vivo*. ○, liver GT activity; ×, liver UDPGA content. The capacity of salicylamide glucuronide formation *in vivo* is expressed as the percentage of the salicylamide glucuronide (mg. of corresponding free salicylamide) excreted in urine to the total salicylamide (mg. of corresponding free salicylamide) excreted in the same urine.

function. The liver GT activity also appeared to be decreased, as a whole, in accordance with the increase in serum GO-T activity which was considered as

reflecting liver-cell necrosis, although this relationship was not apparent in acute viral hepatitis. The similar relationship between the liver GT activity and serum GP-T activity was also observed. There was no evident relationship between the liver GT activity and serum β-glucuronidase activity. Hippuric acid test also failed to correlate with the GT activity or the UDPGA content of the liver tissue.

The relation between the glucuronide formation *in vivo* as measured by the urinary excretion of salicylamide glucuronide after administration of 1 g. of salicylamide and the GT activity or the UDPGA content of the liver tissue is indicated in the same Fig. 6. The capacity of the glucuronide formation *in vivo* was expressed as a ratio of the urinary excreted salicylamide glucuronide to the urinary excreted total salicylamide. In all of the cases examined no free urinary salicylamide was detected. There was a positive correlation between the glucuronide formation *in vivo* and the GT activity of the liver tissue. From the data given in Fig. 6 the correlation coefficient was calculated to be 0.65 with a level of significancy below one per cent. The degree of the decrease in the glucuronide formation *in vivo* was, however, small compared with that in the GT activity of the liver. There was no significant correlation between the glucuronide formation *in vivo* and the UDPGA content of the liver tissue.

DISCUSSION

The necrosis or hydropic swelling of the parenchymal liver cells caused a decrease in the liver GT activity to some extent in viral hepatitis as a whole. However, this was not apparent in acute viral hepatitis, because the decrease in the GT activity was more remarkable in the later stage of the recovery as revealed by the clinical course, the histological findings, and the liver function tests. In other words, in the earlier stage of the recovery from acute viral hepatitis, the reduction in the liver GT activity was not considerable regardless of the fact that evident necrosis and degeneration were observed histologically in the liver. In this connection, it was considered that the diminution or impairment in the parenchymal liver cells, resulting from necrosis or degeneration, caused a reduction in the GT activity of the liver tissue on one side, and on the other side these parenchymal liver injuries might even activate indirectly the liver GT in the earlier stage of acute viral hepatitis. This was partially supported by the fact that there was an increase in the liver GT activity as in the case with chronic colitis. Accordingly, it seemed probable that the liver GT activity might rather increase in some cases of acute viral hepatitis in the early stage of the disease. However, it was also supposed that the liver GT activity would result in a profound decrease even in the early stage of acute viral hepatitis, if

excessive diminution in the parenchymal liver cells was involved as a result of extensive liver-cell necrosis, because a marked decrease in the liver GT activity was observed in the case with massive necrosis and extensive connective tissue proliferation in the liver histology.

The degree of the decrease in the capacity of the glucuronide formation *in vivo* as measured by the urinary salicylamide glucuronide excretion was small compared with that of the reduction in the liver GT activity. This was considered to be attributed to the fact that the formation of salicylamide glucuronide *in vivo* occurred in competition to the formation of other conjugates of salicylamide[36] and also to the assumption that the velocity of the *in vivo* production of UDPGA in the liver might also be a rate-limiting factor for the glucuronide formation *in vivo* other than the GT activity of the liver. The assumption was substantiated to some extent by the following results: 1. Liver UDPGA content failed to correlate with the salicylamide glucuronide formation *in vivo*. 2. The amount of UDPGA used for the conjugation of salicylamide was far greater compared with the whole liver UDPGA content estimated from the UDPGA content of the liver biopsy tissue. 3. The *in vivo* formation of salicylamide glucuronide appeared to be accelerated by an intravenous administration of 500 to 1000 ml. of 5 per cent glucose[36].

The result obtained by MAROGG and WEGMANN[11] that the ratio of serum glucuronic acid to serum bilirubin was decreased in the patient with liver damage and the result indicated by MONOBE[13] that the glucuronide portion of the ester-type direct-reacting bilirubin in the urine of the jaundiced patient with severe liver injury was reduced could be elucidated to some extent from the result of the present study that liver impairments caused a reduction in the liver GT activity and subsequently a decrease in the capacity of glucuronide formation *in vivo*. BILLING[14] and SCHACHTER[15] reported that in chronic cases of viral hepatitis bilirubin monoglucuronide was increased in a relatively large amount as compared with the diglucuronide. This evidence was pertinent with the present observation that the decrease in the liver GT activity was significant in chronic viral hepatitis and in the later stage of the recovery from acute viral hepatitis. However, from the fact that in different liver impairments the alterations in liver GT activity for bilirubin and phenolphthalein glucuronide formations differed from each other[37], it was considered unreaseonable to infer the alteration in bilirubin glucuronide formation from the alteration in 4-MU glucuronide formation. It was also apparent that the alteration in the human liver GT could not be inferred from the results in animal experiments, because in a similar liver injury the alteration in liver GT activity was different in animal species[38]. COHN and BONDY[5], BARNIVILLE and MISK[7], and SCHARNOFF[10] demonstrated the decrease in the capacity of glucuronide formation in the patient with liver cirrhosis

by loading various compounds as glucuronide receptor and by estimating the urinary excreted corresponding glucuronides. This result could be substantiated by the present observation that both GT activity and UDPGA content of liver tissue were profoundly reduced in the patients with cirrhosis.

SUMMARY

The liver glucuronyl transferase (GT) activity and uridine diphosphate glucuronic acid (UDPGA) content in the patients with viral hepatitis were determined using 4-methyl umbelliferone (4-MU) as a glucuronide receptor. The results were as follows:

1. In acute viral hepatitis, the decrease in the GT activity was more remarkable in the later stage of the recovery. In chronic viral hepatitis, the GT activity was decreased in accordance with the increase in the degree of liver injury. Liver UDPGA content was significantly reduced only in postnecrotic cirrhosis.

2. The decrease or injury in the parenchymal liver cells caused a decrease in the liver GT activity. These quantitative reductions in the liver parenchyme were not the only factor for the alteration in the GT activity of the liver. The results of the present study suggested an involvement of a qualitative change in the liver GT activity in human liver injuries, especially in the early stage of acute viral hepatitis; namely, there might be even an activation of the liver GT other than the reduction resulting from the decrease in the liver parenchyme.

3. The decrease in the liver GT activity correlated significantly with the decrease in the salicylamide glucuronide formation *in vivo*, while the alteration in the liver UDPGA content failed to correlate with that in the glucuronide formation *in vivo*. It was suggested that the velocity of *in vivo* UDPGA production rather than the UDPGA content of the liver was as important a rate-limiting factor for the glucuronide formation *in vivo* as the liver GT activity.

ACKNOWLEDGEMENT

The author is greatly indebted to Drs. Y. Shimada and Y. Higuchi for their technical assistance in needle liver biopsy under direct vision on peritoneoscopy in the present study. This work was supported in part by a grant from the Tokyo Biochmical Research Institute.

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