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Enzymatic Studies of Glucuronide Formation in Impaired Liver III. Effects of Carbon Tetrachloride and Ectromelia Virus Infection on Liver Glucuronide Formation in Mouse

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Abstract

Glucuronide formations by mouse liver homogenate in several liver impairments were studied by using 4-methyl umbelliferone as a glucuronide receptor. The results were as follows : 1. Subcutaneous or intraperitoneal administration of carbon tetrachloride to the mouse produced a significant increase in the liver glucuronyl transferase activity 12 or 24 hours after the treatment regardless of histological and enzymatic evidences of liver-cell necrosis. This increase was not attributed to the increase in the 'activator' of glucuronide formation but to the increase in the enzyme activity itself. 2. In Ectromelia virus mouse hepatitis, the glucuronyl transferase activity of the liver tissue was markedly reduced in severe cases. In moderate or milder cases, a slight increase in the activity was observed in a few of them in the early stage of the disease, and the activity was significantly decreased on the recovery in all of the cases which survived. 3. In the early stage of carbon tetrachloride injury when the glucuronyl transferase activity of whole mouse liver was increased and the decomposition of uridine diphosphate glucuronic acid by the liver tissue was also enhanced, the glucuronide formation in vivo was rather increased. It was thus considered that the whole liver glucuronyl transferase activity rather than the uridine diphosphate glucuronic acid content was responsible for the glucuronide formation in vivo as a rate-limiting factor.

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ENZYMATIC STUDIES OF GLUCURONIDE FORMATION IN IMPAIRED LIVER

III. EFFECTS OF CARBON TETRACHLORIDE AND ECTROMELIA VIRUS INFECTION ON LIVER GLUCURONIDE FORMATION IN MOUSE

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Glucuronide conjugation, an important mechanism of detoxication, is known to occur primarily in the liver and involves a microsomal enzyme, glucuronyl transferase (GT), and uridine diphosphate glucuronic acid (UDPGA)[1,2,3,4]. It would be probable that hepatocellular impairments might produce alterations in the GT activity and UDPGA metabolism in the liver, resulting in a derangement in the glucuronide formation *in vivo*. Although these alteratons have been elucidated to some extent in animal experiments, those in human liver are still lacking in evidence especially in early stages of liver disease[5] because of the limitation in obtaining liver tissue at these stages.

In order to infer these alterations in early stages of human liver impairments from the results in experimental liver injuries, the present study was made on the GT activity and UDPGA metabolism of mouse liver in various acute liver injuries. Mice were selected as an experimental animals because of the fact that the histological changes of liver tissue which were similar to those in various stages of human viral hepatitis could be produced by Ectromelia virus infection in mice[6].

MATERIALS AND METHODS

Experimental liver injuries with carbon tetrachloride were induced by the following two methods. In one experimental group, adult male mice, each weighing 15 to 20 g., fed on the Oriental compressed diet for mouse were injected subcutaneously with carbon tetrachloride (0.05 ml. 20 per cent carbon tetrachloride in olive oil per g. body weight). In another group, similar mice were injected intraperitoneally with carbon tetrachloride (0.0125 ml. 20 per cent carbon tetrachloride in olive oil per g. body weight). Ectromelia virus hepatitis was induced by injecting intraperitoneally 0.1 ml, of Ectromelia virus suspen-

sion diluted in varying degrees from the original liver Ectromelia virus emulsion (LD 50 : 10^7-fold dilution) into each of the mice, which were divided into three groups according to the dilution of the virus : 10^0-, 10^4-, and 10^8- fold respectively. Throughout the experiment, five of these animals were sacrificed simultaneously as one group at each of various stages of liver impairments following these treatments. The animal was weighed and killed by bleeding from the femoral artery. The blood was thus processed for serum. A small piece of the liver, approximately 50 mg., was resected and weighed accurately by a torsion balance as quickly as possible, actually within 30 seconds, and immediately transferred to an ice-cold 5 ml. teflon homogenizer. As a suspending medium, ice-cold, alkaline, isotonic potassium chloride[7] was added to the tissue to yield a 2.5 per cent homogenate. Homogenization was performed in the ice water at a speed of 600 r. p. m. for a certain period of time, depending on the amount of the tissue to be homogenized[8]. The GT activity of the homogenate thus prepared was determined according to a modification[8] of the method of ARIAS[9] using 4-methyl umbelliferone (4-MU) as a glucuronide receptor. In this assay, UDPGA as the ammonium salt (90 per cent pure) obtained from Sigma Chemical Company was used. The amount of the 4-MU glucuronide formed with the endogenous UDPGA contained in the homogenate used as GT source was also determined by using the same system, except the addition of UDPGA[8], as that for the determination of the GT activity. The β-glucuronidase activity of the homogenate was determined using p-nitrophenyl glucuronide as a substrate[10]. After weighing the residual liver, the liver was histologically examined. The serum was studied for the activity of other enzymes, such as glutamic oxaloacetic transaminase (GO-T)[11], glutamic pyruvic transaminase (GP-T)[12], and β-glucuronidase[10]. When all of these serum enzyme activities were to be determined, an equal amount of the serum of each of the five mice simultaneously killed as one group was pooled, and the pooled serum was used for the study, because the available amount of serum obtained from one mouse was limited.

The 4 MU glucuronide formation *in vivo* was estimated according to the following assay method. Mice were each injected intraperitoneally with 2 mM 4-MU solution (0.1 ml. per g. body weight), and subsequent 4-hour urine sample was collected. This urine sample was diluted to a final volume of 30 ml. with distilled water. To 0.2 ml. aliquot of the diluted urine, 1.3 ml. of 0.1 M Tris buffer, pH 7.0, containing 26 units of bacterial β-glucuronidase was added. A similar sample without the addition of β-glucuronidase served as a control. Both samples were incubated for 60 minutes at 37°C. After the incubation, 3.0 ml. of 0.2 M glycine buffer, pH 10.42, was added to the hydrolyzed urine sample and the fluorescence was determined against the fluorescence of standard quinine sulfate solution. The amounts of unconjugated 4-MU and its glucuronide both

excreted in the urine were obtained respectively from the fluorescence in the control mixture and from the increase in fluorescence following the enzymatic hydrolysis[8].

RESULTS

Alterations in the activities of liver and serum β-glucuronidase, serum GO-T and GP-T, the ratio of whole liver weight to body weight, and the histological findings of liver tissue following the single subcutaneous injection of carbon tetrachloride are summarized in Table 1. Alterations in the liver GT activity

Table 1. Serum and liver enzyme activities, liver size, and histological findings in acute liver injury in mice following subcutaneous injection of carbon tetrachloride

Hours after CCl_4 Injection	0	12	24	48	96	192
Enzyme activities						
Liver β-glucuronidase (μg./g./hr.)	4920 ±520	5920 ±720	6040 ±960	7207 ±1120	7200 ±1520	6840 ±560
Serum β-glucuronidase (μg./dl./hr.)*	1210	4330	9070	5070	1310	1430
Serum GO-T (μg./0.1 ml./hr.)*	78	858	2016	380	160	119
Serum GP-T (μg./0.1 ml./30 min.)*	10	384	2099	245	34	13
$\frac{\text{Whole liver weight}}{\text{Body weight}} \times 100$ (%)	5.86 ±0.29	5.92 ±0.69	6.36 ±0.94	6.50 ±1.17	7.47 ±1.02	6.04 ±0.41
Histological findings						
Degeneration of liver cells	−	++～+++	++～+++	++～+++	+～++	±
Necrosis of liver cells	−	−～±	+～++	+～++	±～+	−～±
Regeneration of liver cells	−	−	±～+	+～++	+～++	+
Cell infiltrations	−	−	−	±～+	+～++	+

Each value is expressed in mean ± standard deviation in 5 individual determinations. *, activity in the pooled serum from 5 individual sera.

and the 'endogenous' 4-MU glucuronide formation in the same liver injury are illustrated in Fig. 1. Following the subcutaneous administration of carbon tetrachloride, extensive degeneration and centrolobular necrobiosis of liver cells were demonstrated in 12 hours, and the necrosis in the central zone was apparent in 24 hours. In 48 hours inflammatory cell reactions were observed in the necrotic areas, and in 96 hours the necrotic liver cells were replaced by inflammatory cells. In this stage signs of regeneration of liver cells were distinct, and in 192 hours the cellular patterns of the liver were almost normal. At the time of histologically overt hepatocellular necrosis of the liver tissue, 24 hours after the injection, the activity of serum enzymes demonstrated the maximum. The activity tended to decline in the stage of cellular reaction, 48 hours, and returned to normal 192 hours after.

The liver GT activity expressed on wet weight basis increased significantly

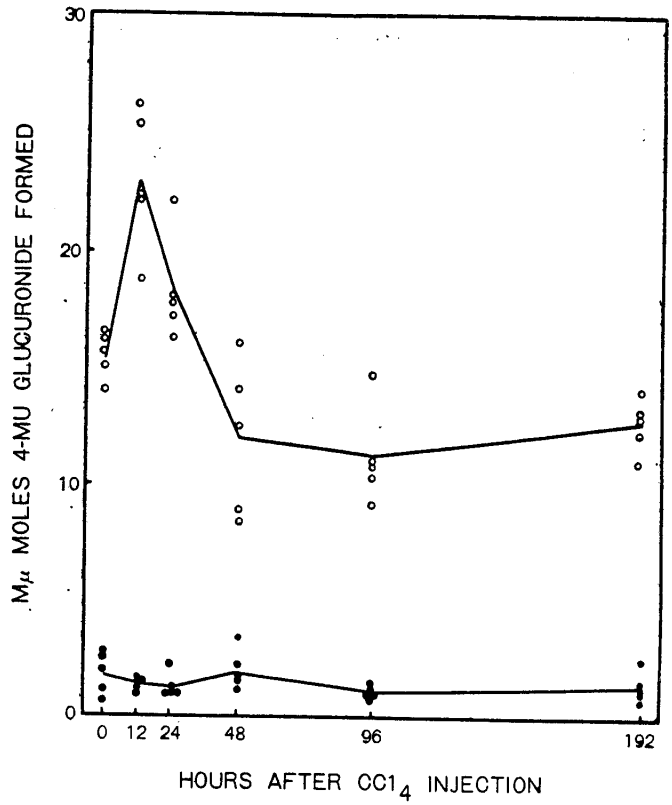

Fig. 1. Effect of acute liver injury by subcutaneous carbon tetrachloride injection on mouse liver GT activity and 'endogenous' 4-MU glucuronide formation. ○, liver GT activity expressed in mμ moles 4-MU glucuronide formed per 10 mg. wet liver weight per 10 minutes: ●, mμ moles 4-MU glucuronide formed 'endogenously' by liver homogenate (equivalent to 10 mg. liver weight).

12 hours after the subcutaneous administration of carbon tetrachloride and tended to decline thereafter. In 96 hours the activity was the minimum and slightly lower than that before the administration. It then appeared to return towards the normal, although the recovery of the activity was not complete even 192 hours later. The injection of carbon tetrachloride also produced a slight increase in the size of liver in 96 hours, and thus the GT activity of the whole liver at this stage was not significantly reduced. GT activity of mouse liver tissue was not affected by the injection of a similar dose of olive oil alone. The 'endogenous' 4-MU glucuronide formation by the liver homogenate used as GT source appeared to be slightly depressed 12 and 24 hours after the carbon tetrachloride administration. The β-glucuronidase activity of the liver tissue was slightly increased in 96 hours, when the histological signs of liver-cell regenera-

tion were apparent; hence the alteration in liver β-glucuronidase activity failed to correlate with that in the GT activity or the 'endogenous' 4-MU glucuronide formation of the liver tissue. When the mice in 96 hours after the injection of carbon tetrachloride were reinjected with carbon tetrachloride in a similar way, they demonstrated, 12 hours after the second injection, also an increase in the GT activity. The increase was almost equal to that noted 12 hours after the initial injection, although the increase in the serum enzyme activities at this time was less.

The results of a similar experiment in case of the single intraperitoneal injection of carbon tetrachloride are indicated in Table 2 and Fig. 2. It was noticed

Table 2. Serum and liver enzyme activities, liver size, and histological findings in acute liver injury in mice following intraperitoneal injection of carbon tetrachloride

Hours after CCl_4 Injection	0	12	24	48	96	192
Enzyme activities						
Liver β-glucuronidase (μg./g./hr.)	4920 ±680	4240 ±150	4760 ±800	4800 ±400	6600 ±1360	5520 ±800
Serum β-glucuronidase (μg./dl./hr.)*	1400	4820	16800	3360	1490	1470
Serum GO-T (μg./0.1 ml./hr.)*	70	1940	5280	2090	93	81
Serum GP-T (μg./0.1 ml./30 min.)*	10	1062	3842	1280	12	11
$\frac{\text{Whole liver weight}}{\text{Body weight}} \times 100\,(\%)$	5.47 ±0.27	6.76 ±0.63	6.33 ±0.93	7.19 ±0.68	7.68 ±0.67	6.46 ±0.29
Histological findings						
Degeneration of liver cells	−	+～卄	卄	卄～卅	+～卄	−～±
Necrosis of liver cells	−	±	卄～卅	卅	+～卄	±
Regeneration of liver cells	−	−	−～±	±～+	+	±
Cell infiltrations	−	−	−	−～±	−～+	±～+

Each value is expressed in mean ± standard deviation in 5 individual determinations. *, activity in the pooled serum from 5 individual sera.

that a consistent and more severe parenchymal liver damage was induced by the intraperitoneal administration as compared with that by the subcutaneous route. Namely, following the intraperitoneal injection, central necrosis of the liver tissue was apparent as early as in 12 hours, the necrotic area was extended to the peripheral zone in 24 hours, and the marked parenchymal derangements characterized by submassive necrosis were demonstrated in 48 hours. The serum enzyme activities 24 hours after the intraperitoneal injection were two to three times those at the same stage after the subcutaneous injection. The histological and enzymatic alterations produced by the intraperitoneal administration of carbon tetrachloride, however, appeared to return towards the normal as rapidly as those by the subcutaneous administration.

The alteration in the liver GT activity following the intraperitoneal carbon

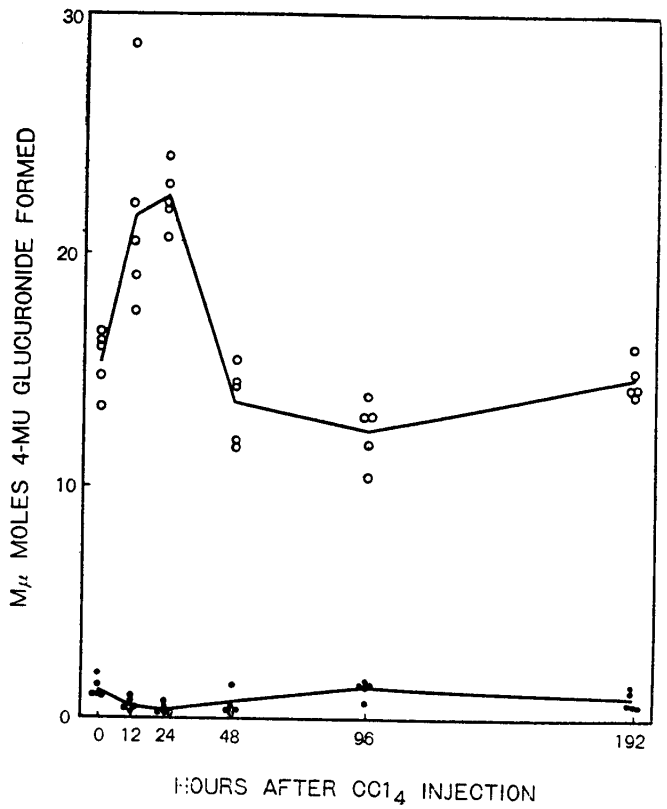

Fig. 2. Effect of acute liver injury by intraperitoneal carbon tetrachloride injection on mouse liver GT activity and 'endogenous' 4-MU glucuronide formation. ○, liver GT activity expressed in mμ moles 4-MU glucuronide formed per 10 mg. wet liver weight per 10 minutes; ●, mμ moles 4-MU glucuronide formed 'endogenously' by liver homogenate (equilvalent to 10 mg. liver weight).

tetrachloride injection was essentially similar to that following the subcutaneous injection, although the maximum GT activity was demonstrated in 24 hours, when the liver-cell necrosis was evident. The reduction in the 'endogenous' 4-MU glucuronide formation in 12 and 24 hours after the intraperitoneal administration was profound compared with that after the subcutaneous route.

A boiled liver extract was prepared from the liver which was obtained from a mouse 12 hours after the intraperitoneal injection of carbon tetrachloride, when the GT activity of the liver was markedly increaed. The activating effect of this boiled liver extract on the 4-MU glucuronide formation by normal mouse liver homogenate was compared with that of the boiled extract prepared from a normal mouse liver. There was no significant difference in the effect between these two boiled extracts, and thus the evidence that the increase in the GT

activity following the carbon tetrachloride treatment might be due to the increase in 'activator'[13] was not obtained.

The results of a similar experiment in Ectromelia virus mouse hepatitis are indicated in Table 3 and Fig. 3. In case of the inoculation of original liver

Table 3. Serum and liver enzyme activities, liver size, and histological findings in acute liver injury in mice following inoculation of Ectromelia virus

Dilution of Ectromelia Virus	1	10^0	10^4		10^8		
Days after Virus Inoculation	0	3	5	6	9	11	15
Enzyme activites							
Liver β-glucuronidase (μg. /g. /hr.)	4360 ±200	2720 ±720	3960 ±320	3640 ±560	4560 ±560	5400 ±680	4720 ±400
Serum GO-T (μg. /0.1 ml. /hr.)	65 ±6	786 ±510	103 ±26	936 ±691	94 ±41	156 ±44	83 ±14
Serum GP-T (μg. /0.1 ml. /30 min.)	10 ±2	198 ±137	20 ±10	290 ±253	19 ±15	32 ±15	16 ±5
$\frac{\text{Whole liver weight}}{\text{Body weight}} \times 100(\%)$	5.96 ±0.30	6.46 ±0.88	5.90 ±0.54	6.80 ±0.39	5.98 ±0.32	5.82 ±0.27	6.28 ±0.43
Histological findings							
Degeneration of liver cells	−	−	±～卄	卄～卅	±～卄	+～卄	±～+
Necrosis of liver cells	−	−～卄	−～±	+～卄	−～+	+	+
Regeneration of liver cells	−	−	−～±	±	−～±	+～卄	±～+
Cell infiltrations	−	−	−	−	−～±	±	−～±

Each value is expressed in mean ± standard deviation in 5 individual determinations.

emulsion of Ectromelia virus (10^0-fold dilution), the mice died of the disease 2 to 3 days after. Microscopic examination of the liver tissue of the sick mice 3 days after the inoculation revealed various degrees of severe parenchymal liver damage characterized by central and mid-zonal focal necroses or submassive necrosis frequently with hemorrhage. Both the GT activity and the 'endogenous' 4-MU glucuronide formation in these mouse liver tissues were markedly reduced.

The mice inoculated with 10^4-fold diluted Ectromelia virus died in 6 days. The liver histology of the sick mice 6 days after the inoculation revealed that there were degenerations and focal necroses mainly in the central zone and mid-zone. At this stage serum GO-T and GP-T activities were markedly increased, while the liver GT activities were almost within the normal range except for a few cases with slight increase. On the other hand, the alteration in the GT activity in the mice within an incubation period, 5 days after the inoculation, was small compared with that in the sick mice and was almost within the normal range.

In case of the inoculation of 10^8-fold diluted Ectromelia virus, initial evidences of the illness were observed nearly 9 days after. Histological changes

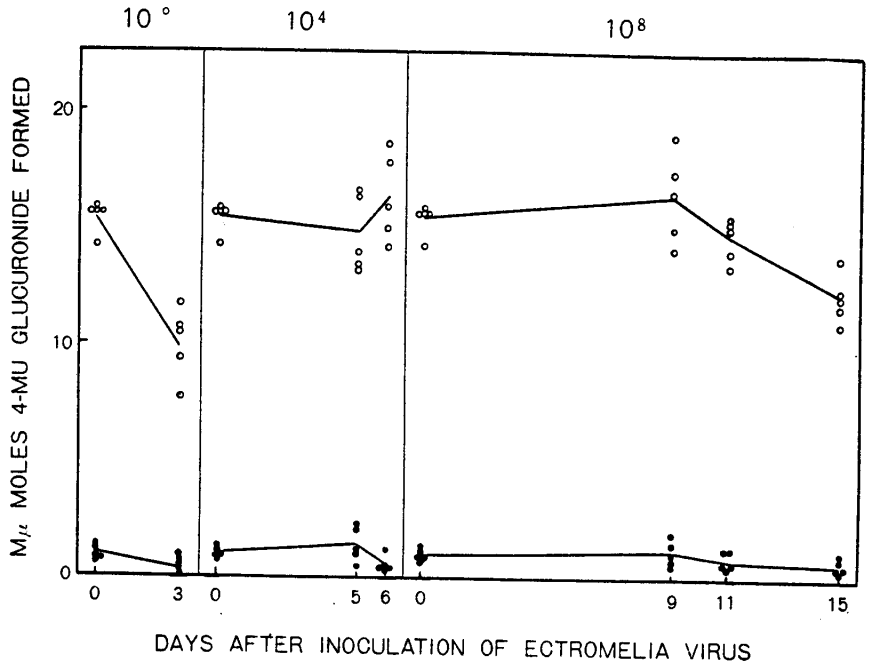

Fig. 3. Effect of acute liver injury by Ectromelia virus infection on mouse liver GT activity and 'endogenous' 4-MU glucuronide formation. Dilution of Ectromelia virus; 10°, 10^4, and 10^8. ○, liver GT activity expressed in mμ moles 4-MU glucuronide formed per 10 mg. wet liver weight per 10 minutes; ●, mμ moles 4-MU glucuronide formed 'endogenously' by liver homogenate (equivalent to 10 mg. liver weight).

in the liver of these mice were slight but characteristic of the virus hepatitis. At this stage a slight increase in the GT activity was observed in a few cases. However, in the stage of the recovery, 11 to 15 days after, the GT activity appeared to be rather decreased to some extent.

The outline of preliminary and tentative experiments for the estimation of 4-MU glucuronide formation *in vivo* were as follows. When normal mice were intraperitoneally injected with 4-MU solution (0.1 ml. per g. body weight) in a different concentration of 0 to 2 mM, the amount of 4-MU glucuronide excreted into the following 4-hour urine of the mice was directly proportioned to the amount of 4-MU administered. Consequently, with these amounts of 4-MU admistered, every process, such as transfer of the injected 4-MU to the site of glucuronide conjugation, formation of 4-MU glucuronide, removal of the formed glucuronide from the site, and its excretion into urine, appeared not to be the rate-limiting step for the 4-MU glucuronide formation *in vivo*. In addition, the results of a similar experiment using 2mM 4-MU solution indicated that the amount of 4-MU glucuronide excreted in each of the first and the second 2-hour

urines was almost equal, and far less amount of free 4-MU was excreted in both urines. In this connection, the amount of 4-MU glucuronide excreted in the 4-hour urine following the 4-MU administration was considered as representing the velocity of the 4-MU glucuronide formation *in vivo* mainly in the mouse liver.

Alterations in the velocity of 4MU glucuronide formation *in vivo* following the subcutaneous injection of carbon tetrachloride are illustrated in Fig. 4. In

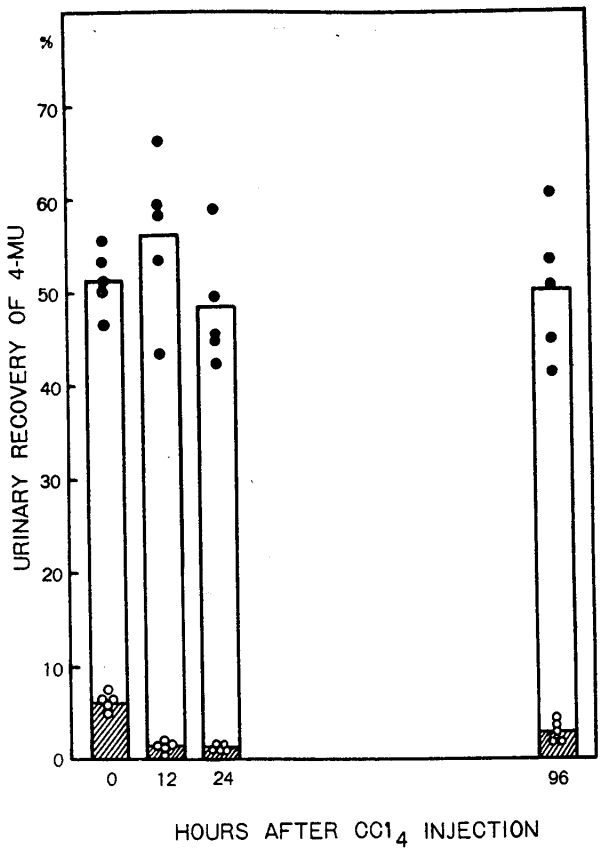

Fig. 4. Effect of acute liver injury by subcutaneous carbon tetrachloride injection on the 4-MU glucuronide formation *in vivo* in mice. Values are indicated in per cent 4-hour urinary recovery of the 4-MU administered as its glucuronide and unconjugated 4-MU (percentage was calculated in terms of mμ moles). The velocity of 4-MU glucuronide formation *in vivo* is expressed in per cent 4-hour urinary recovery of the 4-MU as 4-MU glucuronide. ● and |▬▬■|, 4-MU glucuronide plus unconjugated 4-MU ; ○ and ■, unconjugated 4-MU; |▬▬|, 4-MU glucuronide (the velocity of 4-MU glucuronide formation *in vivo*).

12 hours after the carbon tetrachloride administration, the amount of 4-MU glucuronide excreted in the 4-hour urine was increased as compared with that in

normal control, and that of free 4-MU was decreased. This indicated that the velocity of the 4-MU glucuronide formation *in vivo* in the liver-injured mice in this stage was apparently increased. Even in later stages, 24 and 96 hours after, no reduction in the velocity of 4-MU glucuronide formation *in vivo* was demonstrated.

DISCUSSION

Subcutaneous administration of carbon tetrachloride to the mouse produced degeneration and necrosis of liver cells; nevertheless, it caused a significant increase to occur in the GT activity of the liver tissue at the early stage of the liver injury. In case of the intraperitoneal administration of carbon tetrachloride of one-fourth in the amount as compared with that in the subcutaneous injection, the alteration in the liver GT activity was essentially similar to that produced by the subcutaneous route regardless of the fact that the parenchymal liver damage was more severe than that in the case of the subcutaneous administration. It was noteworthy that the GT activity of liver tissue was increased and the maximum at 24 hours when the liver-cell necrosis was evident and extensive and the elevation in the serum enzyme activities was the maximum. This increase in the mouse liver GT activity at the early stage of carbon tetrachloride poisoning could not be accounted for from the results of ISSELBACHER's work[14] indicating that the reduction in hepatic glucuronide-conjugating capacity of guinea pig induced by carbon tetrachloride was due to a quantitative change in the microsomal GT activity. Although it seemed probable that the increase in the liver GT activity in the present study might be attributed to the increase in the 'activator' of glucuronide formation, such an evidence was not obtained. Therefore, the increase in the GT activity of the mouse liver injured acutely with carbon tetrachloride was considered to be due to rather a qualitative alteration in the mouse liver GT. Although the nature of the microsomal GT of rat liver is known to be different from that of guinea-pig liver[15], there is another report that an acute carbon tetrachloride injury also caused a reduction in the rat liver GT activity[16]. Consequently, it was supposed that the mouse liver GT might further differ in some point from that of rat or guinea-pig liver, and thus the mouse liver GT was affected in quite a different way even by a similar poisoning. On the other hand, the mouse liver GT activity was markedly depressed in case of the severe Ectromelia virus hepatitis. Accordingly, it was clarified that the alteration in mouse liver GT activity was also different, depending on the kind and degree of liver impairment. From these results, it was concluded that the alteration in the GT activity of impaired human liver could not be inferred from the results in experimental liver injuries of animals.

The amount of the 'endogenously' formed 4-MU glucuronide was related

both to the amount of the endogenous UDPGA contained in the homogenate used as GT source and to the amount of the UDPGA decomposed by the homogenate during the incubation and was not affected by the GT activity of the homogenate used. The amount (mμ moles) of the 'endogenously' formed 4-MU glucuronide by normal mouse liver homogenate was indicated to be equivalent to the amount (mμ moles) of UDPGA contained in the homogenate[8]. The decrease in the 'endogenous' 4-MU glucuronide formation in 12 or 24 hours after the carbon tetrachloride injection was considered from the results of previous experiments[8] in part to be resulting from the increased decomposition of endogenous UDPGA by the homogenate during the incubation; hence it was apparent that the UDPGA-decomposing activity of mouse liver in these stages was increased, although the point of whether the decrease in the liver UDPGA content *per se* was involved could not be inferred. POGELL and LELOIR[15] suggested that the activation of the rat liver microsomal GT by uridine diphosphate N-acetylglucosamine might partially be caused by inhibition of UDPGA breakdown. However, this was not a satisfactory means of elucidating the increased GT activity of mouse liver tissue in 12 or 24 hours after the carbon tetrachloride treatment, since the GT activity of mouse liver homogenate in these stages even increased regardless of the fact that the decomposition of UDPGA by the homogenate was evidently enhanced.

The velocity of 4-MU glucuronide formation *in vivo* in carbon tetrachloride-injured mouse was significantly increased in the early stage of the impairment, when the GT activity of the liver tissue was elevated and the decomposition of UDPGA was also increased, and it was not depressed thereafter. This was pertinent with the evidence that the whole liver GT activity of the carbon tetrachloride-poisoned mouse was not significantly reduced during the course of the injury. The amount of endogenous UDPGA contained in mouse liver was far less than that to be used for the formation of 4-MU glucuronide in the presence of an excess amount of UDPGA, and thus it was possible to postulate that the amount of UDPGA present in the liver might be a rate-limiting factor in the glucuronide formation *in vivo*. On the contrary, the evidences described above indicated that the whole liver GT activity rather than the UDPGA content was responsible for the glucuronide formation *in vivo* as a rate-limiting factor. This was also supported by the fact that the mouse liver GT could be saturated with low concentrations of UDPGA as might be the case in the liver tissue. The amount of UDPGA used for the *in vivo* formation of 4-MU glucuronide in 4 hours was calculated far in excess to that of the whole liver UDPGA as calculated from the amount of UDPGA contained in the homogenate. In this connection, it was further considered that the turnover rate of UDPGA in the mouse liver might be sufficiently large and that the *in vivo* production of UDPGA might not

be significantly impaired even in the carbon tetrachloride-injured liver.

SUMMARY

Glucuronide formations by mouse liver homogenate in several liver impairments were studied by using 4-methyl umbelliferone as a glucuronide receptor. The results were as follows:

1. Subcutaneous or intraperitoneal administration of carbon tetrachloride to the mouse produced a significant increase in the liver glucuronyl transferase activity 12 or 24 hours after the treatment regardless of histological and enzymatic evidences of liver-cell necrosis. This increase was not attributed to the increase in the 'activator' of glucuronide formation but to the increase in the enzyme activity itself.

2. In Ectromelia virus mouse hepatitis, the glucuronyl transferase activity of the liver tissue was markedly reduced in severe cases. In moderate or milder cases, a slight increase in the activity was observed in a few of them in the early stage of the disease, and the activity was significantly decreased on the recovery in all of the cases which survived.

3. In the early stage of carbon tetrachloride injury when the glucuronyl transferase activity of whole mouse liver was increased and the decomposition of uridine diphosphate glucuronic acid by the liver tissue was also enhanced, the glucuronide formation *in vivo* was rather increased. It was thus considered that the whole liver glucuronyl transferase activity rather than the uridine diphosphate glucuronic acid content was responsible for the glucuronide formation *in vivo* as a rate-limiting factor.

ACKNOWLEDGEMENT

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