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THE EFFECT OF CARBON TETRACHLORIDE ON THE ENZYMATIC HYDROLYSIS OF CELLULAR TRIACYLGLYCEROL IN ADULT RAT HEPATOCYTES IN PRIMARY MONOLAYER CULTURE

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Abstract—The effect of carbon tetrachloride on the intracellular hydrolysis of triacylglycerol and on the activity of acid triacylglycerol lipase was investigated with primary cultured rat hepatocytes. It was found that the concentration of the precursors in the medium did not affect the time course of the synthesis, secretion and the hydrolysis of triacylglycerol, and that carbon tetrachloride significantly suppressed the hydrolysis of intracellular triacylglycerol and the activity of acid triacylglycerol lipase with a concomitant accumulation of triacylglycerol. The results indicate a possibility that the triacylglycerol accumulation in the cultured rat hepatocytes caused by carbon tetrachloride might be mediated by the suppression of lysosomal acid triacylglycerol lipase activity in addition to the suppression of the secretion of triacylglycerol.

The fatty degeneration induced in the liver by carbon tetrachloride (CCl_4) poisoning appears to be related to an impairment of movement of triacylglycerol (TG) as very low density lipoprotein (VLDL)† [1, 2]. Although the inhibitory effect of CCl_4 on the secretion of TG has been confirmed by several studies with a suspension of isolated rat hepatocytes [3–7], the molecular mechanism responsible for the inhibition of TG secretion remains obscure. Recently, it was demonstrated with isolated hepatocytes that the bioactivation of CCl_4 at the cytochrome P-450 locus is required for the inhibition of TG secretion [8]. While the experimental model using isolated hepatocyte suspension, in fact, allows direct analysis of the intracellular transport of lipoprotein and its discharge outside the cell, it was recognized that the function in the cell membrane of freshly isolated hepatocytes has been greatly impaired by the collagenase digestion [9]. The damage is practically repaired when the isolated cells are cultured as monolayers for 24 hr. From this point of view, it was desired to build up a suitable model with the cultured hepatocytes in which the effect of CCl_4 on the lipoprotein metabolism can be investigated for prolonged period.

In the previous study [10], we have established a new *in vitro* experimental model, i.e. the adult rat hepatocytes in primary monolayer culture in which the level of cytochrome P-450 was retained at the level comparable to the intact liver. In this model, CCl_4 was able to cause inhibition of TG secretion throughout the incubation time, and the accumulation of TG in the hepatocytes was observed 6 hr after exposure. It was suggested, on the other hand, that the inhibition of TG secretion was not an exclusive mechanism for the accumulation of TG, since the suppression in TG secretion did not necessarily result in the accumulation of TG and, even if the accumulation occurred, the amounts of TG in the hepatocytes did not balance with the extent of the suppression of the secretion.

The level of TG in the liver is presumed to be regulated by not only the rates of the synthesis but also the removal by secretion from the previous observation [11], whereas the possibility of the involvement of the enzymatic hydrolysis of TG was unknown. Therefore, the effects of CCl_4 on the hydrolysis of TG and on the lysosomal acid TG lipase activity (EC 3.1.1.3), which is believed to be responsible for the hydrolysis of TG containing within lipoprotein particles [12, 13], were investigated with the monolayer cultured hepatocytes. Furthermore, the effect of varying the concentration of the precursors, glycerol and fatty acid, in the medium on the synthesis and secretion as well as the hydrolysis of TG were investigated to evaluate their possible involvement in the development of TG accumulation in the cultured hepatocytes.

MATERIALS AND METHODS

Animals. Male Wistar rats (180–200 g) were housed in wire-bottom cages and allowed free access to food and water. The animals were treated with 80 mg/kg sodium phenobarbital in Dulbecco's phosphate buffered saline (PBS) intraperitoneally once

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† Abbreviations used: BSA, bovine serum albumin; DME medium, Dulbecco's modified Eagle's medium; NCS, newborn calf serum; PBS, Dulbecco's phosphate buffered saline; TG, triacylglycerol; VLDL, very low density lipoprotein.

each day for 2 days. They were sacrificed for hepatocyte isolation 24 hr after the last injection.

Chemicals and media. [2-^{3}H]Glycerol (spec. act. 1.0 Ci/mmol) and [1-^{14}C]oleic acid (spec. act. 56 mCi/mmol) were purchased from Amersham, U.K. [Carboxyl-^{14}C]triolein (spec. act. 111.8 mCi/mmol) was from NEN Research Products, U.S.A. *Clostridium histolyticum* collagenase (Type 1), Soybean trypsin inhibitor (Type-IIS), bovine pancreas insulin, β-glycerophosphate (disodium salt, Grade I), and egg yolk phosphatidylcholine (Type XI-E) were the products of Sigma Chem. Co., U.S.A. Bovine serum albumin (BSA, fatty acid poor, fraction V) was from Miles Scientific, U.S.A. Powdered Dulbecco's modified Eagle's (DME) medium and Eagle's minimum essential medium were from Nissui Seiyaku Co., Ltd., Tokyo. Newborn calf serum (NCS) was from Irvine Scientific, U.S.A. Penicillin–streptomycin solution, Fungizone and 0.4% trypan blue solution were purchased from Gibco Laboratory Co., U.S.A. Dexamethasone (Dexa-Sheroson) was from Shering AG, Berlin. 2-Methyl-1,2-di-3-pyridyl-1-propanone (metyrapone) was from Aldrich Chem. Co., U.S.A.

Preparation of cultured hepatocytes. Cells were dissociated from phenobarbital-treated rats by perfusion with Ca^{2+}- and Mg^{2+}-free Hanks' solution containing 0.5 mM ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid, then with 0.05% collagenase and 0.005% trypsin inhibitor dissolved in Hanks' solution as described previously [10]. Dissociated cells were suspended in Eagle's minimum essential medium and hepatocytes were sedimented at 50 *g* for 1 min. The washings of hepatocytes with the medium were repeated three times by centrifugation. Cell number was counted by a hemocytometer, and the viability of the cell, as assessed by the exclusion of trypan blue, was usually more than 90%. Cells were plated in 60 mm (2.8×10^6 cells/4 ml of medium) or in 35 mm (9.8×10^5 cells/1.4 ml of medium) Falcon plastic culture dishes and cultured in DME medium supplemented with 10% NCS, 0.5 mM metyrapone, 100 nM insulin, 1 μM dexamethasone, Fungizone (0.25 mg/l), penicillin (10^5 units/l), and streptomycin (100 mg/l) under 5% CO_2/air at 37°. The medium was replaced 5 hr after the inoculation to remove unattached cells, and then cultured further for 19 hr.

Incubation of the cultured hepatocytes with labeled precursor. After the hepatocyte monolayer was prepared, the medium was replaced with fresh DME medium containing antibiotics, fatty acid (sodium salt, 0.1–1.0 mM) bound to 1% fatty acid poor BSA, and [^{3}H]glycerol (100 mCi/mmol, 0.1–10 μCi). The incubation was carried out at 37° in a CO_2-incubator for the indicated time in the presence or absence of CCl_4 (2 mM) which were prepared by the sonication for 10 min in the fatty acid free control medium. In some experiments, 100 nM insulin and 1 μM dexamethasone were added to the incubation medium to make the condition identical with the previous study [10]. The incubation was terminated by placing the dishes on ice and the medium was transferred into screw-capped tubes to centrifuge at 700 *g* for 5 min. The monolayer was washed twice with PBS and the cells were recovered by scraping with a rubber policeman into screw-capped tubes with 1 ml of PBS. The cells were stored at −20° overnight. After thawing, the cells were shaken vigorously and then frozen at −20° again for 2 hr. After thawing and shaking again, the aliquots (0.1 ml) were submitted to protein determination as described by Lowry *et al.* [14]. In some experiments, [^{14}C]sodium oleate was used instead of [^{3}H]glycerol as a labeled precursor. For the enzyme assays hepatocytes were recovered from a 60 mm culture dish with 1 ml of 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4) and 1 mM EDTA, then subjected to freeze-thawing twice as described above. The homogenate of hepatocytes thus obtained was used for a source for the assay of enzyme activity.

Lipid analysis. Lipid was extracted from medium and cells according to the method of Bligh and Dyer [15]. The organic phase was washed with methanol–0.9% NaCl (1:1) containing 1% glycerol, and then with 50% methanol twice. The lipid extract was evaporated with a stream of nitrogen and applied on a thin layer chromatoplate which was then developed with *n*-hexane–diethyl ether–acetic acid (80:20:1). The plates were briefly exposed to iodine vapor and the spots corresponding to triacylglycerol (TG) were scraped into small columns. TG was eluted with 2 ml of chloroform–methanol (2:1) 6 times and TG content was determined by the method of Van Handel and Zilversmit [16], using tripalmitin as a standard. The radioactivity incorporated into TG was determined in 10 ml toluene scintillation mixture.

Enzyme assays. Acid TG lipase was determined according to the method of Burrier and Brecher [17] with slight modifications. The emulsion was prepared from equimolar amounts (2.35 μmol) of [carboxyl-^{14}C]triolein (spec. act. 0.85 mCi/mmol, 2 μCi) and egg yolk phosphatidylcholine in 2 ml of a solution containing 0.1 M NaCl, 0.01 M Tris–HCl buffer (pH 7.4) by sonication for 4 min with Sonic Dismembrator (Artex) at a power setting of 50. The incubation mixture contained 50 mM sodium acetate (pH 4.4), 2.5 mM 2-mercaptoethanol, 0.5 mM EDTA, 50 μl labeled substrate and 50 μl of hepatocyte homogenate, in a final volume of 250 μl. After the incubation at 37° for 20 min, the reaction was terminated by the addition of 4 ml of benzene–chloroform–methanol (1.0:0.5:1.2, v/v/v) containing 0.1 mM unlabeled oleic acid as a carrier. An 0.6 ml aliquot of 0.3 M NaOH was then added, and the resulting suspension was mixed for 15 sec followed by centrifugation at 1000 *g* for 10 min to separate the phases. The free fatty acid was then determined by counting a 1 ml aliquot of the upper phase in 10 ml of ACS-II in a scintillation counter.

Acid phosphatase activity was determined by measuring the hydrolysis of β-glycerophosphate. Incubation mixture contained 50 mM β-glycerophosphate, 75 mM sodium acetate buffer (pH 4.8), 0.5 mM EDTA, 2.5 mM 2-mercaptoethanol and 0.2 ml of hepatocyte homogenate in a final volume of 0.4 ml. After incubation at 37° for 30 min the reaction was stopped with 2 ml of 10% trichloroacetic acid and the mixture was centrifuged at 1000 *g* for 10 min. Inorganic phosphate in 1 ml aliquot of the supernatant was determined according to the method of Fiske and Subbarow [18].

Table 1. Effect of glycerol concentration on the incorporation of [^{14}C]oleic acid into triacylglycerol in the cultured hepatocytes

Glycerol concentration (μM)	Addition of hormones	[^{14}C]Oleic acid incorporation into cellular TG ($\times 10^3$ dpm/mg cell protein)
0.33	−	7.53 ± 0.83
3.3	−	8.06 ± 0.43
3.3	+	9.09 ± 0.79
33.0	−	10.26 ± 0.49
330.0	−	10.88 ± 0.43
3300.0	−	11.38 ± 1.06

After the monolayers were obtained by culturing the hepatocytes for 24 hr in DME medium containing 10% NCS and 0.5 mM metyrapone, the medium was changed to serum-free DME medium containing various concentration (0.33 μM–3.3 mM) of unlabeled glycerol and 1 mM of [^{14}C]oleic acid (spec. act. 72.4 mCi/mol) bound to fatty acid poor serum albumin, and then the hepatocytes were further incubated for 3 hr. The radioactivity incorporated into cellular TG at 3 hr in the absence or presence of hormones (100 nM insulin and 1 μM dexamethasone) was determined as described in the text. The values are the mean ± SD of four determinations.

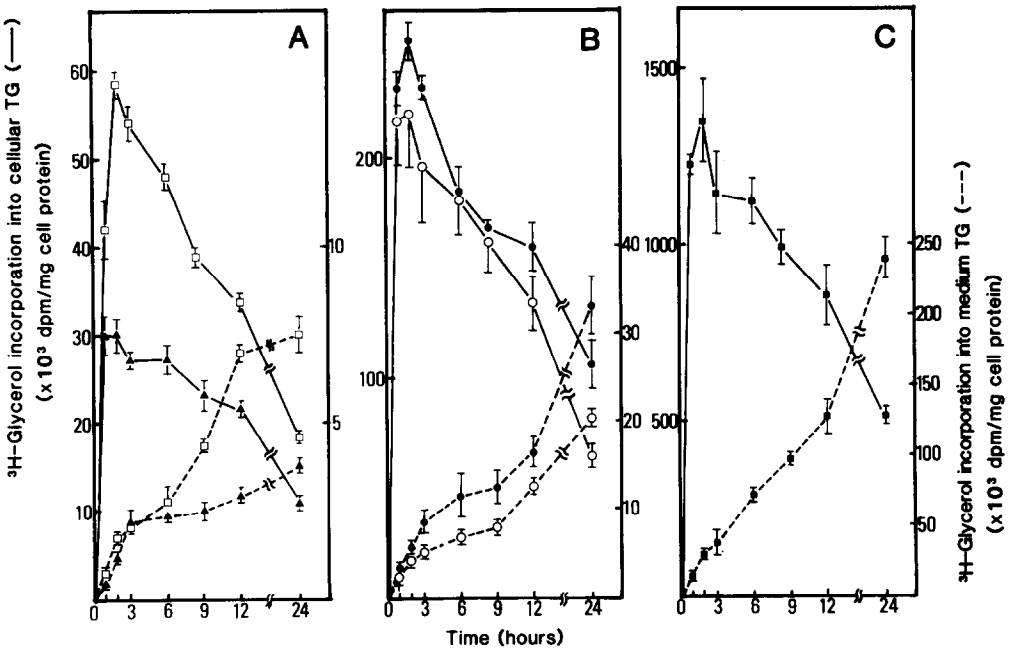

Fig. 1. Effect of [^{3}H]glycerol concentration on the incorporation into triacylglycerol in the cultured hepatocytes. After the monolayers were obtained by culturing the hepatocytes for 24 hr in DME medium containing 10% NCS and 0.5 mM metyrapone, the medium was changed to serum-free DME medium containing various concentration (0.33–33 μM) of [^{3}H]glycerol (spec. act. 100 mCi/mmol) and 1 mM oleic acid bound to fatty acid poor BSA, and then the hepatocytes were further incubated for 24 hr. The radioactivity incorporated into cellular and medium TG was shown by solid and dotted line, respectively. Each point represents the mean ± SD of three determinations. Glycerol concentration: ▲, 0.33 μM; □, 0.66 μM; ●, 3.3 μM; ○, 3.3 μM with 100 nM insulin and 1 μM dexamethasone; ■, 33 μM.

RESULTS

Effect of glycerol concentration on the incorporation of [^{3}H]*glycerol into cellular and medium TG*

The time course of the incorporation of [^{3}H]glycerol into TG is shown in Fig. 1 at the [^{3}H]glycerol concentration of 0.33, 0.66, 3.3 and 33 μM. The radioactivity in the cellular TG increased rapidly until 1 or 2 hr at oleic acid concentration of 1 mM, then decreased at a relatively constant rate. The time course of the radioactivity in the cellular TG showed a similar pattern at any concentration of glycerol. Treatment with hormones did not affect significantly the radioactivity incorporated into cellular and medium TG. It was recently demonstrated that the presence of 1 μM dexamethasone and 36 nM insulin decreased the incorporation of [^{3}H]-glycerol

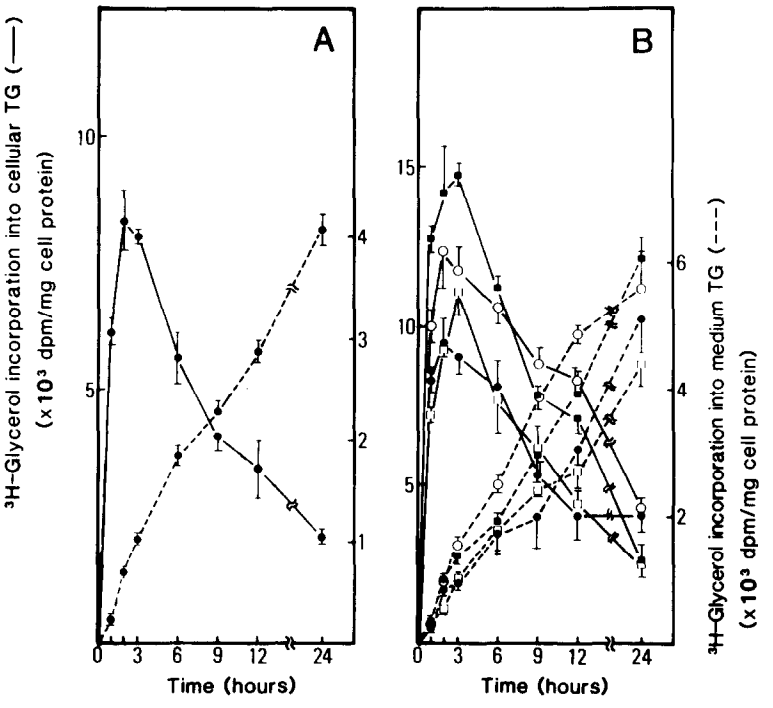

Fig. 2. Effect of 0.1 mM fatty acids bound to bovine serum albumin on the incorporation of [^{3}H]glycerol into triacylglycerol in the cultured hepatocytes. After the monolayers were obtained as described in the legend to Fig. 1, the medium was changed to serum-free DME medium containing 3.3 μM [^{3}H]glycerol (1 μCi) and fatty acid poor BSA (A) or 3.3 μM [^{3}H]glycerol (1 μCi) and 0.1 mM of various species of fatty acid bound to fatty acid poor BSA (B), and then the hepatocytes were further incubated for 24 hr. The radioactivity incorporated into cellular and medium TG was shown by solid and dotted lines, respectively. Each point represents the mean ± SD of three determinations. (A) ●, fatty acid poor BSA; (B) ■, palmitic acid; □, stearic acid; ●, oleic acid; ○, linoleic acid.

into cell TG [19]. The difference is presumed to arise from the experimental conditions. The radioactivity incorporated into medium TG was increased gradually with time, but the level was one-tenth to one-twentieth of radioactivity of cellular TG during the incubation, although the difference became small at the late period of the incubation. The similar time course of the incorporation was observed by Nossen *et al.* [20] in the cultured hepatocytes with the 25 μM [^{3}H]glycerol and 1 mM oleic acid. The rate of incorporation of [^{3}H]glycerol into cellular TG was directly proportional to the labeled glycerol concentration in the medium. However, when [^{14}C]oleic acid was used instead of [^{3}H]glycerol as radioactive precursor, no significant difference was observed in the incorporation of the labeled oleic acid into TG in spite of the varying concentration of glycerol in the medium (Table 1). These results indicate that the rate of TG synthesis was not changed by the alteration of glycerol concentration (0.33 μM–3.3 mM), and that the specific activity of the intracellular pool of glycerol changed proportionally with the concentration of [^{3}H]glycerol in the medium.

Effect of fatty acid concentration on the incorporation of [^{3}H]*glycerol into cellular and medium TG*

In the absence of exogenous fatty acid, the incorporation rate of [^{3}H]glycerol into TG was very low at a glycerol concentration of 3.3 μM (Fig. 2A), as compared with that in the presence of 1 mM oleic acid (see Fig. 1B). With 0.1 mM of different species of fatty acid bound to BSA, the incorporation of labeled glycerol into both cellular and medium TG was slightly increased. However, the time course of the incorporation was not changed substantially by the addition of various species of fatty acids from that with BSA alone (Fig. 2B). Figure 3 shows the effects of increasing the fatty acid concentration in the medium on the incorporation of labeled glycerol. The radioactivity in both cellular and medium TG was increased according to the concentrations of oleic acid (Fig. 3A), indicating that fatty acid enhanced the rate of the synthesis and secretion of TG. Although 1.0 mM linoleic acid also caused the activation of TG synthesis and secretion (Fig. 3B), the rate was relatively lower than that of 1 mM oleic acid. Since the concentration of labeled glycerol was constant at 3.3 μM in this experiment, it should be noted that radioactivity incorporated into both cellular and medium TG was proportional to the rate of the synthesis and secretion of TG. Irrespective of the different rates of TG synthesis, the time course of the increase and decrease of the radioactivity in cellular TG was not changed at all from those of above experiments (Figs 1 and 2).

Effects of CCl_4 *on the rate of hydrolysis of cellular TG and on acid TG lipase activity*

The incubation was carried out with the hepatocytes, which had been prelabeled with [^{3}H]glycerol

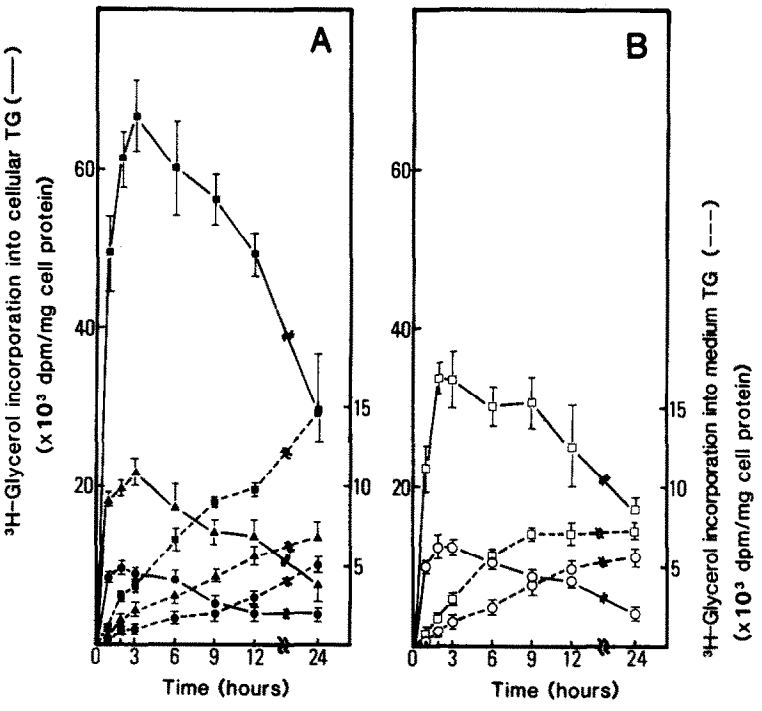

Fig. 3. Effect of concentration of fatty acid in the medium on the incorporation of [^{3}H]glycerol into triacylglycerol in the cultured hepatocytes. After the monolayers were obtained as described in the legend to Fig. 1, the medium was changed to serum-free DME medium containing 3.3 μM [^{3}H]glycerol (1 μCi) and fatty acids bound to fatty acid poor BSA, and then the hepatocytes were further incubated for 24 hr. The radioactivity incorporated into cellular and medium TG was shown by solid and dotted line, respectively. Each point represents the mean ± SD of three determinations. (A) Oleic acid concentration: ●, 0.1 mM; ▲, 0.5 mM; ■, 1.0 mM. (B) Linoleic acid concentration; ○, 0.1 mM; □, 1.0 mM.

for 3 hr, in the medium free from the precursors. The effects of CCl_4 on the hydrolysis of cellular TG and activity of acid TG lipase were investigated concomitantly. The incorporation of [^{3}H] glycerol into both cellular and medium TG increased for initial 3 hr, then the radioactivity in cellular TG in control hepatocytes decreased immediately after the medium was changed with precursor-free medium (Fig. 4A). The decrease in the radioactivity in cellular TG would be owing to the hydrolysis and the secretion of labeled TG. However, the latter contribution is presumed to be minor in this experimental system, because the amount of labeled TG secreted into the medium was far smaller than that decreased intracellularly. On the other hand, the radioactivity of cellular TG in CCl_4-treated hepatocytes still continued to increase for 1 hr after removing the precursors from the medium. Furthermore, the rate of the hydrolysis of labeled TG was significantly lower in CCl_4-treated hepatocytes than control hepatocytes for 3–9 hr. The cellular TG accumulated actually in CCl_4-treated hepatocytes after 6 hr (Fig. 4B). Thus, the lipid accumulation was observed though CCl_4 did not suppress significantly the secretion of labeled TG. This time course of the accumulation of TG in CCl_4-treated hepatocytes was consistent with that of the previous study [10].

The time course of the activity of acid TG lipase and acid phosphatase as a lysosomal marker enzyme was shown in Figs 5A and 5B, respectively. The acid TG lipase in control hepatocytes was relatively constant with some fluctuation after the medium was changed, while the activity was suppressed gradually in the hepatocytes treated with CCl_4. On the contrary, acid phosphatase in control hepatocytes was not significantly different from that of CCl_4-treated hepatocytes until at least 12 hr. The ratio of the activity of acid TG lipase to that of the acid phosphatase in CCl_4-treated hepatocytes (4.8–6.7) was always smaller than that in control (6.7–8.1), indicating that acid TG lipase is more sensitive against CCl_4 toxicity than acid phosphatase. Overall the results suggest the possibility that CCl_4 suppressed the activity of lysosomal acid TG lipase, resulting in the inhibition of hydrolysis of TG. The accumulation of cellular TG was demonstrated in this experimental model to be derived in part from the suppression of intracellular hydrolysis of TG.

DISCUSSION

The level of hepatic TG has been reported to be regulated, in some cases, by the hydrolysis of intracellular TG. TG accumulated within the hepatocytes was demonstrated to be hydrolyzed during the recovery of several pathological states [21], and by hormonal control in chick cultured hepatocytes [22]. However, the subcellular site and the mode of

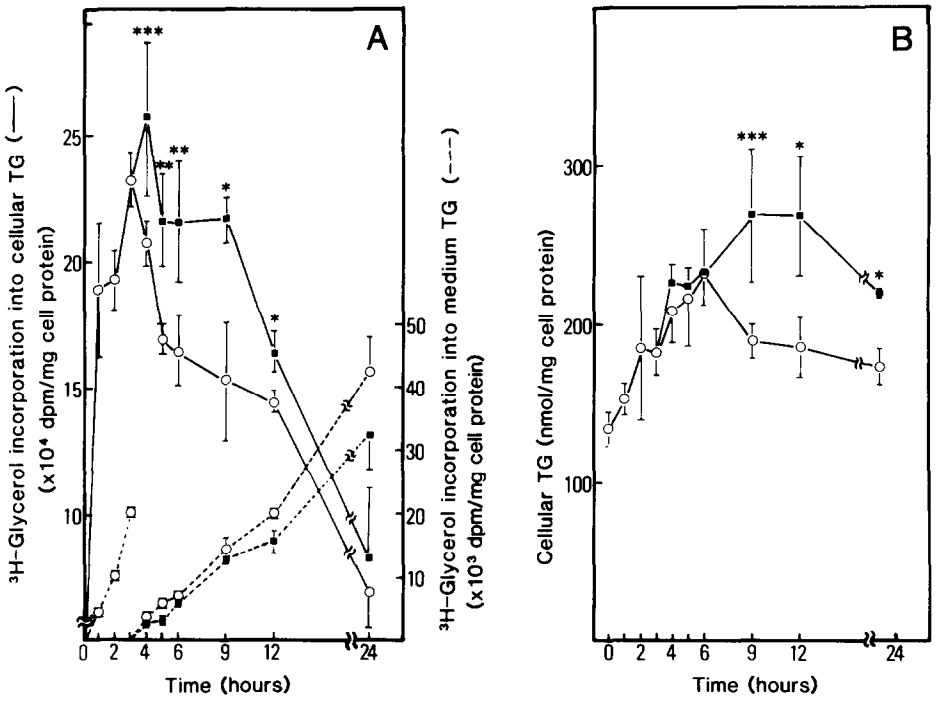

Fig. 4. Effect of CCl_4 on the hydrolysis and the content of cellular triacylglycerol. After the monolayers were obtained as described in the legend to Fig. 1, the medium was changed to serum-free DME medium containing 3.3 μM [^{3}H]glycerol (1 μCi) and 1 mM oleic acid bound to fatty acid poor BSA. At 3 hr after the beginning of the incorporation with labeled precursor, medium was removed and changed again to serum-free DME medium containing no precursor for TG synthesis, and then the hepatocytes were further incubated for 21 hr in the presence or absence of 2 mM CCl_4. (A) The radioactivity incorporated into cellular and medium TG was shown by solid and dotted line, respectively. (B) Content of cellular TG at indicated time was determined as described in the text. Each point represents the mean ± SD of four determinations: ○, control; ■, 2 mM CCl_4. Values which are significantly different from that of control are indicated by: *$P < 0.01$; **$P < 0.025$; ***$P < 0.05$.

regulation of TG hydrolysis were unknown. In the present study, the effects of CCl_4 on the hydrolysis of intracellular TG was investigated by pursuing the change in the radioactivity incorporated into cellular TG and the alteration in acid TG lipase activity.

The maximum incorporation of the radioactivity into cellular TG was always observed during 2–3 hr after the beginning of the incubation in spite of various concentrations of glycerol and fatty acids (Figs 1–3), indicating that the decrease in the radioactivity in cellular TG was not attributable to the reduction of specific activity of the pool of glycerol because of the sufficient supply of [^{3}H]glycerol. The major portion of the decrease in the radioactivity in cellular TG would be due to the enzymatic hydrolysis, although some part of the activity is removed to the medium and the specific activity of labeled precursor would be reduced endogenously. Thus, the rate of TG synthesis was greater than the hydrolysis until 2 hr and thereafter the rate of hydrolysis of cellular TG came to surpass the synthesis in this experimental model. Therefore, the effects of CCl_4 on the hydrolysis of TG and the activity of acid TG lipase were investigated after the preincubation with [^{3}H]glycerol for 3 hr. CCl_4 suppressed considerably the hydrolysis of TG without affecting the rate of the secretion into the medium (Fig. 4A), resulting in the accumulation of TG from 6 hr after the exposure (Fig. 4B). The most likely mechanism for the accumulation of TG in hepatocytes has been shown to be the inhibition of the secretion of TG with no change in TG synthesis [3–8]. However, the quantitative relationship between the inhibition of TG secretion and the accumulation of TG has not been shown. From this point of view, it is noteworthy that the correlation of the accumulation of TG and the suppression of TG hydrolysis was observed in the present study (Figs 4A and 4B).

The effect of CCl_4 on the acid TG lipase was investigated, since lysosomal acid TG lipase is a major intracellular lipase which is believed to hydrolyze TG in lipoprotein particles [12, 13], and Debeer *et al.* [23] suggested that endogenously synthesized TG would be transported into the lysosomes for hydrolysis. Another type of intracellular lipase, a non-lysosomal TG lipase with a neutral pH optimum was not investigated in the present study because it could not be distinct from the hepatic TG lipase which is destined to be secreted outside the hepatocytes [24, 25]. Since the isolation of lysosomal fraction from the cultured hepatocytes was difficult because of the limited amounts of lysosomal protein, we tried to uncover the latent lysosomal activity from the cultured hepatocytes by freeze-thawing,

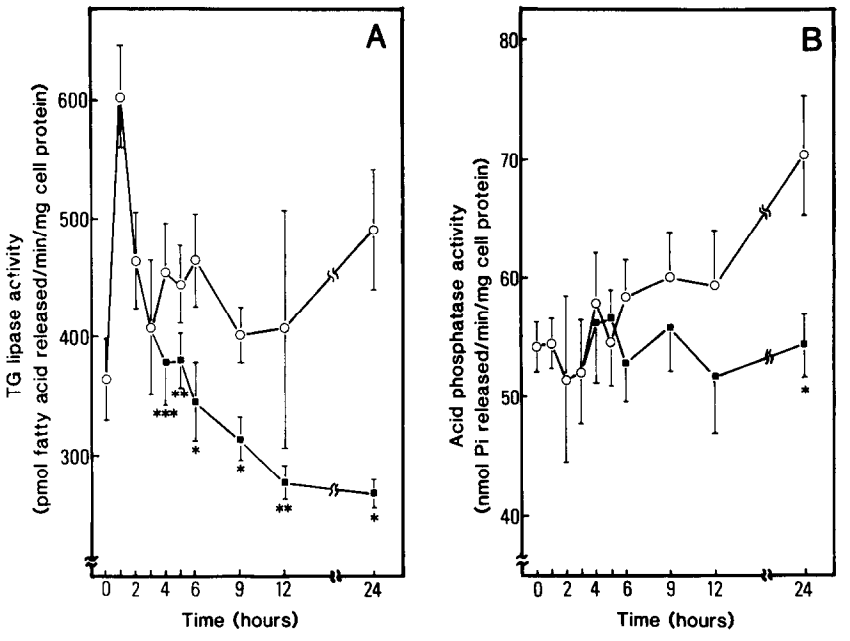

Fig. 5. Effects of CCl_4 on the activities of acid triacylglycerol lipase and acid phosphatase. The condition of the incubation was exactly identical to that described in the legend to Fig. 4. The activities of acid TG lipase (A) and acid phosphatase (B) were assayed as described in the text. Each point represents the mean ± SD of four determinations: ○, control; ■, 2 mM CCl_4. Values which are significantly different from that of control are indicated by: *$P < 0.005$; **$P < 0.01$; ***$P < 0.025$.

sonication, or addition of Triton X-100 [26]. Among these conditions, the maximum activity was obtained from the hepatocytes treated by freeze-thawing as described in the text. The activity was assayed after the incubation of hepatocytes with the precursor-free DME medium, since TG lipase was found to be inhibited by the addition of fatty acids [27]. The suppression of the activity of acid TG lipase by CCl_4 correlated well with the suppression of the hydrolysis of intracellular TG and the accumulation of TG in CCl_4-treated hepatocytes (Figs 4 and 5). These results strongly suggest a possibility that the suppression of acid TG lipase is involved in the development of the accumulation of TG. In this respect, it should be noted that the accumulation of both TG and cholesterol ester in fibroblasts from patients with Wolman's disease and cholesteryl ester storage disease would be caused by the deficiency of lysosomal TG lipase and the cholesteryl esterase (EC 3.1.1.13) [28, 29]. Although the subcellular site of the hydrolysis or the localization of acid TG lipase were not assigned in this experimental model, we noted that the acid TG lipase in the lysosomal fraction prepared by the method of Regab *et al.* [30] was suppressed significantly 3 hr after the i.p. injection with 1 ml/kg CCl_4 to rat (H. Kato and Y. Nakazawa, unpublished work).

Recently, it was found by Coleman and Haynes [31] that there exists another intracellular acid TG lipase in the microsomal fraction which might be differentiated from lysosomal TG lipase. The microsomal TG lipase is proposed to hydrolyze endogenously synthesized TG that accumulates in hepatocytes during various pathological states. On the other hand, the function of acidic subcellular fractions including the trans element of Golgi apparatus as well as the lysosomes was proposed to be essential for the secretion of VLDL [20], although the suppression of TG secretion could not be observed in the present experiment. Therefore, the relevance of the present study could be confirmed by the future investigations concerning the localization of CCl_4-labile acid TG lipase and the relationship between the hydrolysis of TG in the corresponding acidic fraction(s) and the secretion of VLDL.

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