《原 著》

Uptake of ^{67}Ga in the Liver of Rats Treated with CCl_4

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ABSTRACT The uptake of ^{67}Ga citrate was studied in the liver of rats treated with CCl_4 associated with biochemical parameters. Marked elevation of ^{67}Ga uptake in the rat liver after a single administration of CCl_4 was found at 2 to 3 days. The elevated uptake of ^{67}Ga in the liver appeared to be closely correlated with the rate of DNA synthesis and the induction of hepatic glucose-6-phosphate dehydrogenase activity among the biochemical parameters studied in relation to the hepatocellular injury caused by CCl_4.

Pretreatment of CCl_4-treated rats with aminoacetonitrile was effective in lowering both the elevated uptake of ^{67}Ga in the liver and the increased incorporation of $^{35}SO_4$ into the liver mucopolysaccharides induced by CCl_4.

The elevated uptake of ^{67}Ga in the liver of CCl_4-treated rats was also inhibited when protein synthesis was stopped by cycloheximide administration.

Cellulose acetate electrophoresis of crude mucopolysaccharides extracted from the control and CCl_4-treated rats indicated that ^{67}Ga was bound to only heparan sulfate in both groups.

Thus, heparan sulfate appears to play an important role in the elevated uptake of ^{67}Ga in liver injury induced by CCl_4 administration to rats.

Introduction

Recently ^{67}Ga citrate has been increasingly used as a diagnostic agent of imaging a wide range of human cancers and for the detection of inflammatory lesions.[1~2] However, the accumulation mechanism of ^{67}Ga in these tissues is not yet clearly understood,

Our previous study[3] on ^{67}Ga accumulation during hepatocarcinogenesis induced by 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) indicated that elevated accumulation of ^{67}Ga occurred in 2 stages, corresponding to the early and the later stages of this hepatocarcinogenesis, and that this pattern coincided well with the patterns of hepatic γ-glutamyltranspeptidase (γ-GTP) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities. Recent histological and biochemical findings[4~6] during hepatocarcinogenesis induced by 3′-Me-DAB demonstrated that the early elevation of γ-GTP activity might be simply as a reflection of an acute liver lesion caused by the toxic effect of 3′-Me-DAB and that the later elevation coincided with the development of tumor cells. Thus, accumulation of ^{67}Ga might follow the repairing of damaged liver cells and parallel the progress of the carcinogenic process induced by 3′-Me-DAB, suggesting that some common mechanism may be involved. Furthermore, a remarkable change in ^{67}Ga subcellular distribution was found in the $800 \times g$ fraction during hepatocarcinogenesis induced by 3′-Me-DAB. To obtain more information on the mechanism of ^{67}Ga, in the present study we investigated the uptake of ^{67}Ga in the liver of rats treated with CCl_4.

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Materials and Methods

Male Donryu rats, weighing about 170 g and 6 weeks of age, were obtained from Nihon Rat Co., Urawa. The animals were maintained on a basal diet CE-2 (CLEA Japan Inc., Tokyo) and water *ad libitum* for 2 weeks before experiments. A single administration of CCl_4 was given to overnight-fasted rats. A dose of 0.5 ml of 20% CCl_4 in liquid paraffin per 100 g body weight was given intragastrically. Aged-paired control rats were treated similarily with equivalent amounts of liquid paraffin alone. Aminoacetonitrile (AAN) was given by i.p. injection at a dose of 20 mg dissolved in physiological saline per 100 g body weight daily for 6 days before the CCl_4 treatment. Cycloheximide (CYH) was given i.p. injection at a dose of 1 mg per kg body weight at 30 min before the injection of ^{67}Ga citrate.

In order to determine the uptake of ^{67}Ga and biochemical changes after the CCl_4 treatment, the following biochemical analyses were performed for 5 days. Serum alanine aminotransferase (L-alanine: α-oxoglutarate aminotransferase, EC 2. 6. 1. 2) (GPT) activity by the method of Reitman and Frankel,[7] and expressed as Karmen units per ml of serum (KU). Activity of G-6-PDH in liver supernatant was determined as described previously.[6] Hepatic γ-GTP activity in liver microsome fractions was measured by the method of Cameron.[8] These activities were expressed in mU (nmole of the product formed per minutes at 37°) per mg protein. Protein content was determined by the method of Lowry.[9]

Carrier-free ^{67}Ga was supplied by New England Nuclear, Boston, Mass., and diluted with 0.08 M sodium citrate to 50 μCi/ml. The rats were given i. v. injection of ^{67}Ga citrate (10 μCi/animal). After 2 hr, the rats were killed by cutting the carotid arteries under light pentobarbital anesthesia, and the blood was taken for determination of serum GPT activity. The liver was quickly perfused with ice-cold isotonic physiological saline, removed and weighed, and the ^{67}Ga radioactivity was counted with a well-type scintillation counter (Aloka TDC-501). The percentage of the administered radioactivity per gram liver in each group of 5 rats is presented as mean $\pm$ standard deviation at each time interval.

Hepatic DNA synthesis was investigated by giving rats i.p. injection of ^{3}H-thymidine (^{3}H-Tdr) (10 μCi/animal; specific activity 21.5 Ci/mmole) 2 hr before sacrifice. The liver was perfused with ice-cold isotonic physiological saline, removed, blotted, and homogenized in 5 volumes of cold 0.03 M Tris-HCl buffer (pH 7.4). Two ml aliquotes of homogenate were each extracted with 2.5 ml of 10% trichloroacetic acid (TCA). The subsequent isolation of DNA was carried out to the methods of Schmidt and Thannhauser[10] and of Schneider.[11] Radioactivity in DNA fractions was measured in toluene-Triton X-100 (2:1) scintillator after the removal of TCA by washing 3 times with 2 volumes of ether. The results are given as dpm/g liver.

In order to investigate incorporation of $^{35}SO_4$ into liver $800\times g$ mucopolysaccharides (MPS), rats were injected intraperitoneally with 10 μCi of [^{35}S] sulfate (Radiochemical Centre, Amersham, England; specific activity, 2.2 mCi/mmole) 2 hr before sacrifice. The liver was perfused with ice-cold physiological saline and homogenized with 5 volumes of 0.25 M sucrose (pH 7.6). The homogenate was centrifuged at $800\times g$ for 15 min. The precipitates were used for the preparation of crude MPS according to the procedure of Schiller.[12] The radioactivity in MPS fraction dissolved in water was measured in toluene-Triton X-100 (2:1) scintillator. The determination of uronate in the crude MPS was carried out by means of the carbazole reaction as modified by Bitter and Muir.[13] Glucuronic acid (Wako Fine Chemicals Co., Tokyo, Japan) was used as a standard.

Protein synthesis was determined by measuring the incorporation of L-[^{14}C] leucine (5 μCi/animal; specific activity, 280 mCi/mmole) (New England Nuclear, Boston, Mass.) injected intraperiotoneally 20 min before sacrifice, A 20% homogenate in 0.25 M sucrose (pH 7.6) was acidified and precipitate formed was washed with 10% TCA. The acid-insoluble protein residue was dissolved in Protosol (New England Nuclear, Boston, Mass.). The specific radioactivity data are presented as dpm/g liver.

^{67}Ga-binding MPS were identified by cellulose acetate strip electrophoresis. Crude MPS exracted from the CCl_4-treated rat liver by the method as described above were incubated with ^{67}Ga

citrate (50 μCi/ml) in 0.01 M HEPES buffer (pH 6.8) at 37° for 1 hr. The MPS were collected by centrifugation after precipitation with ethanol containing 1% potassium acetate. Precipitated MPS were washed 3 times with ethanol, dissolved in a small volumes of distilled water, and applied to a cellulose acetate strip (Separax, Jōkō Sangō Co., Tokyo, Japan), and electrophoresis was performed under a constant current (0.6 mA/cm) in 0.2 M calcium acetate (pH 7.6). After electrophoresis, strips were stained with 0.5% toluidine blue according to the procedure of Seno.[14] Radioactivity of ^{67}Ga was detected with Packard radiochromatograph scanner (model 7230).

Results

1) ^{67}Ga uptake and Biochemical Changes

^{67}Ga uptake and biochemical changes in hepatocellular injury induced by CCl_4 treatment are shown in Figs. 1 to 3. Serum GPT value increased immediately after the CCl_4 treatment, reaching a maximum within 2 days. ^{67}Ga uptake in the liver was also elevated from the 1st day after treatment of CCl_4, and reached a maximum at the 2nd to 3rd day, but the serum GPT elevation and increase of ^{67}Ga uptake did not occur in parallel (Fig. 1). ^{3}H-Tdr incorporation into DNA rose from the 1st to 2nd day, reaching a maximum at the 3rd day after CCl_4 treatment. On the other hand, hepatic γ-GTP, which has been indentified as a possible positive marker for preneoplastic hepatocytes,[15] reached its peak at 4 days after the CCl_4 treatment (Fig. 2). In addition, hepatic G-6-PDH, an enzyme which is characteristically induced in the process of repair or proliferation of liver cells,[16] was activated from the 1st day, and reached a maximum at 2 to 3 days after CCl_4 treatment (Fig. 3). From these results, it is clear that ^{67}Ga uptake after CCl_4 treatment is closely correlated with DNA synthesis and hepatic G-6-PDH activity.

2) Effect of AAN on Elevated ^{67}Ga Uptake in the liver of CCl_4-treated Rats

AAN, which is known to be a lathyrogenic agent,[17] was tested for inhibitory effect on the elevated uptake of ^{67}Ga in the liver at 2.5 days after CCl_4 treatment. As shown in Table 1, continuous pretreatment with AAN caused a decrease of ^{67}Ga uptake in the liver, though the uptake was still very much greater than that of the control.

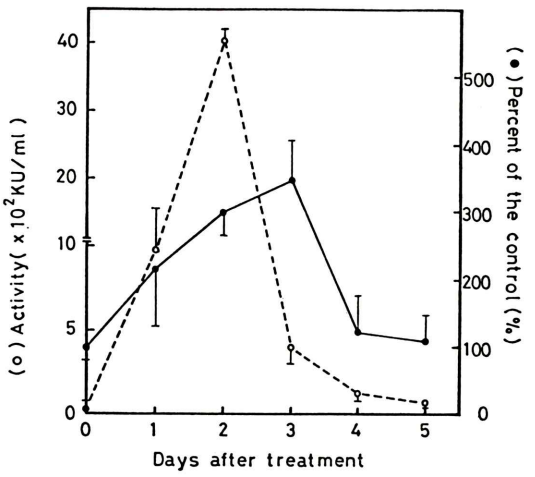

Fig. 1 Time courses of serum GPT activity and ^{67}Ga citrate uptake in rat liver after CCl_4 treatment. A dose of 0.5 m*l* of 20% CCl_4 in liquid paraffin per 100 g body weight was given intragastrically to rats. Solid and dotted lines indicate the mean values (5 rats) of ^{67}Ga uptake (●) and serum GPT activity (○), respectively. Vertical lines represent the standard deviation.

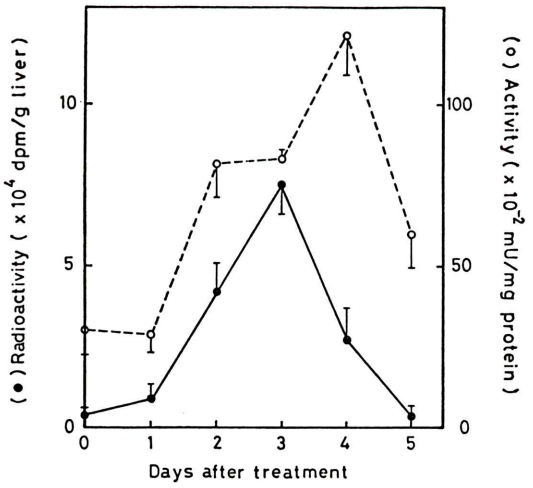

Fig. 2 Time courses of ^{3}H-Tdr incorporation into DNA and γ-GTP activity in rat liver after CCl_4 treatment. ^{3}H-Tdr was given intraperitoneally at a dose of 10 μCi to each rat. Solid and dotted lines indicate the mean value (5 rats) of ^{3}H-Tdr incorporation (●) and γ-GTP activity (○) in rat liver, respectively. Vertical lines represent the standard deviation.

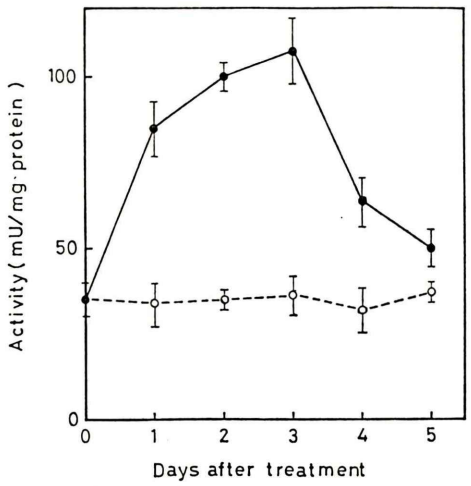

Fig. 3 Level of G-6-PDH activity after CCl_4 treatment. Solid and dotted lines indicate the mean value (5 rats) of hepatic G-6-PDH activity in rats treated with CCl_4 and in the control rats, respectively. Vertical lines represent the standard deviation.

This inhibitory effect on ^{67}Ga uptake was observed in all subcellular fractions, but was particularly significant in the 800×g fraction. The pretreatment with AAN was also effective in inhibiting the remarkable increase of $^{35}SO_4$ incorporation into 800×g crude MPS fractions after CCl_4 treatment. However, the specific activity, $^{35}SO_4$ per uronate, was approximately the same in all groups (Table 2).

3) Effect of CYH on Elevated ^{67}Ga Uptake in the Liver of CCl_4-treated Rats

It has been reported that the incorporation of $^{35}SO_4$ into MPS is greatly inhibited when protein synthesis is stopped by administration of antibiotics such as puromycin.[18] Then, the effect of CYH, a potent inhibitor of protein synthesis,[19] was investigated in CCl_4-treated rats at the 3rd day after the treatment. CYH was effective in lowering the marked increase of ^{14}C-leucine incorporation into the protein fraction of CCl_4-treated rat liver, and also reduced the ^{67}Ga uptake to about one-half of that in CCl_4-treated rat liver (Fig. 4).

4) Identification of ^{67}Ga-binding MPS

Electrophoretograms of crude MPS are shown in Fig. 5. Crude MPS extracted from the livers of the control and CCl_4-treated rats (at the 3rd day) contained at least two to three types of MPS. Two major MPS were dermatan sulfate and heparan sulfate in both groups. Radioelectrophoretogram indicated that ^{67}Ga binds only with heparan sulfate in both groups.

Discussion

The present study indicated that ^{67}Ga uptake in the liver of CCl_4-treated rats is closely correlated with DNA synthesis and hepatic G-6-PDH induction, and that pretreatment of the CCl_4-treated rats with AAN or CYH was effective in lowering the elevated uptake of ^{67}Ga in the liver.

It is well known that hepatic fibrosis occurs as a repair mechanism for necrosis induced by hepatotoxic agents and that accumulation of MPS is seen at the beginning of this pathologic change.[20] With respect to the biochemical changes in hepatic fibrogenesis, Popper et al.[21] and Rubin et al.[22] reported that the DNA content increased at an

Table 1 Effect of aminoacetonitrile (AAN) on ^{67}Ga uptake in the liver of rats treated with CCl_4

	Liver	800×g[a]	Mt[b]	Mc[c]	Sp[d]
Control	9,052± 874[e] (100)	22± 2[f] (100)	78±11 (100)	32± 5 (100)	46± 7 (100)
CCl_4	73,371±11,600 (810)	245±33 (1104)	511±58 (655)	174±30 (544)	300±47 (652)
CCl_4+AAN	34,923± 3,922 (386)	97±12 (446)	292±43 (374)	100± 7 (313)	124±10 (270)

[a] 800×g precipitate. [b] Mitochondrial fraction. [c] Microsomal fraction. [d] 105,000×g supernatant.
[e] cpm/g liver. [f] cpm/mg protein.
Figures in parentheses indicate the relative value with respect to the contol.
The control was arbitrarily taken as 100%.
Each value represents the mean±SD (n=5).

early stage during hepatic fibrogenesis induced by ethionine or CCl_4 simultaneously with an increase of collagen. Schmidt et al.[23] suggested that the pentose cycle (PC) might be a main pathway of glycolysis in hepatic sclerosis. In this study, the time at which the uptake of ^{67}Ga in the liver reached a maximum was also in good accord with the times of peak activation of hepatic G-6-PDH, a key enzyme of PC, and DNA synthesis. These results suggest that the uptake of ^{67}Ga is closely correlated with hepatic fibrogenesis. This view is supported by the fact that AAN, a lathyrogenic agent which inhibits fibrogenesis and the accumulation of acid MPS,[24] significantly reduced the elevated uptake of ^{67}Ga in the liver of CCl_4-treated rats, while the uptake was also inhibited when protein synthesis was stopped by CYH. It is generally accepted that MPS exist *in vivo* as protein-MPS complexes and that following completion of the protein, requisite sugars are added to complete the protein-MPS complexes.[18] This biosynthetic

Table 2 Effect of aminoacetonitrile (AAN) on $^{35}SO_4$ incorporation into mucopolysaccharides in the liver 800×g fraction of rats treated with CCl_4

Group	^{35}S/g DEFLF[a] (cpm)	Uronate/g DEFLE (μmole)	^{35}S/Uronate (cpm/μmole)
Control	15,930 ± 712[b]	376 ± 4	48.9 ±6.2
CCl_4	62,336 ±2,721	1,024 ±127	51.9 ±9.3
CCl_4+AAN	34,361 ±1,806	668 ± 35	46.3 ±7.8

[a] Defatted liver 800×g fraction

[b] Each value represents the mean±standard deviation (n=5).

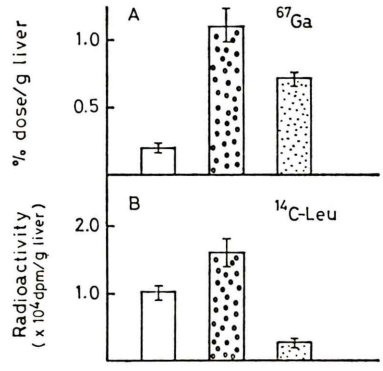

Fig. 4 Effect of cycloheximide (CYH) on ^{67}Ga uptake and protein synthesis in the liver of rats treated with CCl_4. CYH was given to the CCl_4-treated rats (the 3rd day after CCl_4 treatment) by i.p. injection at a dose of 1 mg/Kg body weight 30 min before injection of ^{67}Ga citrate (10 μCi). Protein synthesis was determined by measuring the uptake of L-[^{14}C] leucine (10 μCi) injected i.p. 20 min before sacrifice. ☐ Control; ⁘ CCl_4; ⋰⋱ CCl_4+CYH. Vertical lines represent standard deviation (n=5).

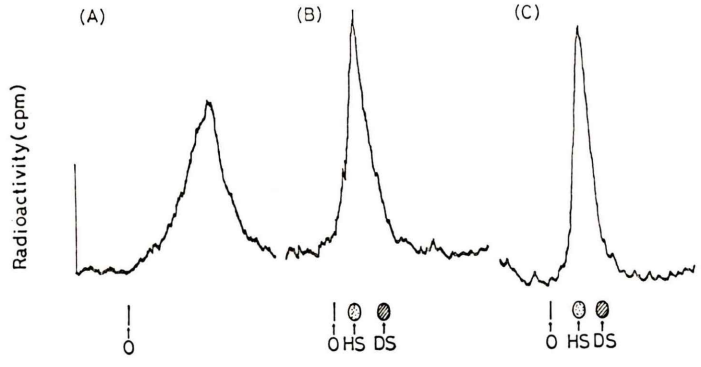

Fig. 5 Cellulose acetate electrophoretogram of crude mucopolysaccharides (MPS) from the livers of control and CCl_4-treated rats. (A) ^{67}Ga citrate only, (B) ^{67}Ga+liver MPS from control rat, and (C) ^{67}Ga+liver MPS from CCl_4-treated rat. O, origin; HS, heparan sulfate; DS, dermatan sulfate.

sequence of protein-MPS complexes seems to be consistent with the result that the elevated ^{67}Ga uptake in the liver of CCl_4-treated rats was reduced when protein synthesis was stopped by CYH *in vivo*. Further studies by cellulose acetate electrophoresis indicated that ^{67}Ga was bound only with liver heparan sulfate *in vitro*. Various workers have reported on the role, the localization and the metabolism of heparan sulfate. Kraemer et al.,[25] Yamamoto et al.,[26] Buonassisi et al.,[27] and Murata et al.[28] have shown that heparan sulfate is located on the cell surface and involved in the binding, and/or transport of ions and positively charged protein. Recently a role has been proposed for heparan sulfate in cell differentiation[29] based, among other data, on the conspicuous presence of this compound in the outer cell coat,[25] together with the fact that Ca^{++} ions are also present in the outer cell-layer in high amounts[30] and are effective ligands for MPS. The localization of heparan sulfate there may account for the marked accumulation of ^{67}Ga in $800 \times g$ fraction reported by Clausen et al.[31]

Radioautographic studies on ^{35}S-sulfate uptake into cartilage have indicated that the localization of MPS synthesis is the Golgi apparatus,[32] while Aronson et al.[33] showed that MPS were degraded in lysosomes. These data may also account for the previous reports that lysosomes are the site of accumulation of ^{67}Ga,[34~37] and that heavy endoplasmic reticulum participates in gallium localization.[38]

On the other hand, Orii et al.,[39] and Hill et al.[40] reported that ^{67}Ga uptake is not related to hepatic cell proliferation in regenerating rat liver after partial hepatectomy. However, the absence of elevated uptake of ^{67}Ga after partial hepatectomy may be due to the fact that the collagen concentration in the residual cirrhotic liver decreased immediately after partial hepatectomy, and values similar to those of normal hepatectominized rats were only obtained after 1 year.[41] Therefore, it appears that ^{67}Ga uptake in liver cells might be related the rapid cell proliferation that characterizes regeneration and repair, together with hepatic fibrogenesis.

Significant increases of MPS have been reported not only in hepatic cirrhosis[42~43] but also in various animal and human tumors.[44~46] Recently, Kupchella et al.[47] have reported that tissue from fast-growing, intermediate, and slow-growing Morris hepatomas exhibited greater glycosaminoglycans levels than did normal liver in the chondroitin sulfate-heparan sulfate fraction. Therefore, heparan sulfate may participate significantly in ^{67}Ga accumulation in tumor cells and inflammatory tissues.

Our present study thus indicates that heparan sulfate might be an acceptor for ^{67}Ga accumulation not only in tumor cells but also in inflammatory lesions. Further studies are necessary to elucidate the nature of this accumulation mechanism.

References

1) Higasi T, Nakayama Y, Murata A, et al: Clinical evaluation of ^{67}Ga-citrate scanning. J Nucl Med **13**: 196–201, 1972
2) Blair DC, Carroll M, Carr EA, et al: ^{67}Ga citrate for scanning experimental staphylococcal abscesses. J Nucl Med **14**: 99–102, 1973
3) Sasaki T, Kojima S, Kubodera A: Changes of ^{67}Ga accumulation in rat liver during hepatocarcinogenesis induced by 3′-methyl—4-dimethylaminoazobenzene. Jap J Nucl Med **19**: in press 1982
4) Iwasaki T, Dempo K, Kaneko A, et al: Fluctuation of various cell populations and their characteristics during azodye carcinogenesis. GANN **63**: 21–30, 1972
5) Taniguchi N, Tsukada Y, Mukuo K, et al: Effect of hepatocarcinogenic azodye on glutathione and related enzymes in rat liver. GANN **65**: 381–387, 1974
6) Kojima S, Hama Y, Kubodera A: Glucose-6-phosphate dehydrogenase and γ-glutamyltranspeptidase activities in the liver during chemically induced hepatocarcinogenesis in rat and mice. Toxicol Appl Pharmacol **60**: 26–32, 1981
7) Reitman S, Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am J Clin Pathol **28**: 56–63, 1957
8) Cameron R, Kellen J, Kolin A, et al: γ-glutamyltranspeptidase in putative premalignant liver cell populations during hepatocarcinogenesis. Cancer Res **38**: 823–829, 1978
9) Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem **193**: 265–275, 1951
10) Schmidt E, Thannhauser SJ: A method for the determination of desoxyribonucleic acid, ribon-

ucleic acid and phosphoproteins in animal tissues. J Biol Chem **161**: 83–89, 1945
11) Schneider WC: Phosphorous compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid of pentose nucleic acid. J Biol Chem **161**: 293–303, 1945
12) Schiller A, Slover GA, Dorfman A: A method for the separation of acid mucopolysaccharides, its application to the isolation of heparin from the skin of rats. J Biol Chem **236**: 983–992, 1961
13) Bitter T, Muir HM: A modified uronic acid carbazole reaction. Anal Biochem **4**: 330–334, 1962
14) Seno N, Anno K, Kondo K, et al: Improved method for electrophoretic separation and rapid quantitation of isometric chondroitin sulfates on cellulose acetate strips. Anal Biochem **37**: 197–202, 1970
15) Farber E: Putative precursor lesions: summary and some analytical consideration. Cancer Res **36**: 2703–2705, 1976
16) Watanabe A, Taketa K: Actinomycin D-insensitive induction of rat liver glucose-6-phosphate dehydrogenase by carbon tetrachloride injury. J Biochem **73**: 771–779, 1973
17) Fiume L: Inhibition by aminoacetonitrile of early lesions induced in the liver of rats by carbon tetrachloride. J Path Bact **83**: 291–293, 1962
18) Telser A, Robinson HC, Dorfman A: The biosynthesis of chondroitin-sulfate protein complex. Proc Natl Acad Sci **54**: 912–918, 1965
19) Threlfall G, Taylor DM: Modification of folic acid induced changes in renal nucleic acid and protein synthesis by actinomycin D and cycloheximide. Eur J Biochem **8**: 591–596, 1969
20) Popper H, Schaffner F, Hutterer H, et al: Parencnymal fibrogenesis: the liver. Ann New York Acad Sci **86**: 1075–1088, 1960
21) Popper H, Paronetto F, Schaffner F, et al: Studies on hepatic fibrosis. Lab Ivest **10**: 265–290, 1961
22) Rubin E, Hutterer E: Hepatic fibrosis. "The connective tissue" edited by Wagner, Williams and Wilkins. p. 142–160, 1967
23) Schmidt E, Schmidt FW: Activäts-Bestimmungen von Enzymen des Energieliefernden Stoffwechsels bei Chronischen Leber—Entzündungen. Klinishe Woehenchrift **36**: 611–619, 1958
24) Sambe K: The 3rd Meeting of the International Association for the Study of the Liver, Liver 1966
25) Kraemer PM: Heparan sulfate of cultured cells. I. Membrane associated and cell-sap species in Chinese hamster cells. Biochemistry **10**: 1437–1445, 1971
26) Yamamoto K, Terayama H: Comparison of cell coat acid mucopolysaccharides of normal liver and various ascites hepatoma cells. Cancer Res **33**: 2257–2264, 1973
27) Buonassisi V, Root M: Enzymatic degradation of heparin-related mucopolysaccharides from the surface of endothelial cell cultures, Biochim Biophys Acta **385**: 1–20, 1975
28) Murata K: Acid glycosaminoglycans in human kidney tissue. Clin Chim Acta **63**: 157–170, 1975
29) Chiarugi VP, Vannucchi S: Surface heparan sulfate as a control element in eukariotic cells: a working model. J. Theor Biol **61**: 459–475, 1976
30) Borle AB: Regulation of cellular calcium metabolism and calcium transport by calcitonin. J Membrane Biol **21**: 124–145, 1975
31) Clausen J, Edeling CJ, Fogh J: ^{67}Ga binding to human serum protein and tumor components. Cancer Res **34**: 1931–1937, 1974
32) Kimata K, Okayama M, Suzuki S: Nascent mucopolysaccharides attached to the Golgi membrane of Chondrocytes. Biochim Biophys Acta **237**: 606–610, 1971
33) Aronson NN, Davidson EA: Catabolism of mucopolysaccharides by rat liver lysosomes in vivo. J Biol Chem **10**: 4494–4499, 1968
34) Swartzendruber DC, Nelson B, Hayes RL: Gallium-67 localization in lysosomal-like granules of leukemic and non leukemic murine tissues. J Natl Cancer Inst **46**: 941–952, 1971
35) Paterson AHG, Yoxall A, Smith I, et al: The concentration of ^{67}Ga and ^{45}Ca in the lactating mammary gland and its relevance to the tumour uptake of ^{67}Ga citrate. Cancer Res **36**: 452–457, 1976
36) Takeda S, Uchida T, Matsuzawa T: A comparative study on lysosomal accumulation of Gallium-67 and Indium-111 in Morris Hepatoma-7316A. J Nucl Med **18**: 835–839, 1977
37) Takeda S, Okuyama S, Takusagawa K, et al: Lysosomal accumulation of Gallium-67 in Morris Hepatoma-7316A and Shionogi mammary carcinoma-115. GANN **69**: 267–271, 1978
38) Samezima H, Orii H: In vivo localization of Ga-citrate in rat liver as determined by cell fractionation with isopycnic ratezonal ultracentrifugation. Eur J Nucl Med **5**: 281–288, 1980
39) Orii H: Tumor scanning with Gallium (^{67}Ga) and its mechanism studied in rats. Strahlentherapie **144**: 192–200, 1972
40) Hill JH, Henry N, Wagner Jr: ^{67}Ga uptake in the regenerating rat liver. J Nucl Med **15**: 818–820, 1974
41) Rabinovici N, Wiener E: Collagen and deoxyribonucleic acid concentrations during regenera-

tion in the hepatectomized cirrhotic liver. Gastroenterology **41**: 251–257, 1961
42) Popper H, Paronetto F, Barka T: PAS positive structures of non-glycogenic character in normal and abnormal liver. A M A Arch Path **70**: 300–313, 1960
43) Calambos JT: Acid mucopolysaccharides (APS) in human cirrhosis. J Clin Invest **42**: 933, 1963
44) Kojima J, Nakamura N, Kanatani M, et al: The glycosaminoglycans in human hepatic cancer. Cancer Res **35**: 542–547, 1975
45) Hopwood JJ, Dorfman A: Glycosaminoglycan synthesis in Wilms' tumor. Pediatr Res **12**: 52–56, 1978
46) Danishefsky ET, Oppenheimer O, Heritier-Watkins Willhite M: Mucopolysaccharides in animal tumors. Cancer Res **26**: 229–232, 1966
47) Kupchella CE, Drake EE, Kennedy J, et al: Tissue and urinary glycosaminoglycan pattern associated with a fast, an intermadiate, and a slow-growing Morris hepatoma. Cancer Res **41**: 419–424, 1981

要　　旨

四塩化炭素投与ラット肝における ^{67}Ga の取り込み

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四塩化炭素 (CCl_4) 投与ラット肝における ^{67}Ga の取り込みを生化学的変化と関連付け検討した．CCl_4 1回投与後，2～3日目で ^{67}Ga の肝への著しい取り込み上昇がみられた．この上昇は肝 DNA 合成および肝 Glucose-6-phosphate dehydrogenase 活性と極めて良い関連性を示した．

CCl_4 投与ラット肝にみられた ^{67}Ga の著しい取り込み上昇は Aminoacetonitrile (AAN) の前処理により顕著に抑制された．また，この上昇は蛋白合成を Cycloheximide (CYH) により止めるとき抑制された．これらの結果より，^{67}Ga とムコ多糖との関連性が示唆されたため，セルロース，アセテート膜電気泳動法により検討すると，Heparan sulfate との結合性が説められた．

以上の結果より，CCl_4 傷害肝における ^{67}Ga の取り込み上昇に Heparan sulfate の関与が示唆された．

Key words: Gallium 67, Liver, CCl_4, Heparan sulfate