Experimental and Clinical Studies on the Intrahepatic Vascular Changes in Chronic Liver Injury

(I) Microscopic Vital Observation of the Mouse Liver in Chronic Carbon-tetrachloride Intoxication

HIROYASU TAMAO

The First Department of Internal Medicine, Okayama University Medical School, Okayama
(Director: Prof. Kiyono Kawai)

Morphological changes of the intrahepatic vascular systems and mechanism of the development of the intralobular porto-hepatic anastomoses in the mouse liver in chronic carbon-tetrachloride intoxication were studied by means of microscopic vital observation and also by a new injection method in living animals with India ink.

Existence of arterial sinusoids and their role in development of the intralobular communications were observed. With the advance of hepatic lesions, a decrease in width of the intrahepatic venous branches, which had occurred firstly in the most distal portions, attained gradually to the proximal divisions.

The manner of the intrahepatic distribution of vascular system and its alteration in the damaged liver are far from being fully understood. There exists a considerable number of literature on the vascular pattern of cirrhotic liver, but little work has been done to study that of the chronic stage preceding cirrhotic stage. The discussion and historical review of the subject are described in the paper published by Hales et al. Concerning to the terminal distribution of hepatic artery, controversy continued as to whether the communication between the terminal artery and terminal portal branches exist, and whether the arterial capillaries penetrate deep into the lobules.

Abnormalities in intrahepatic hemodynamics in chronic hepatitis has been demonstrated from this laboratory. These findings suggest that the morphological changes of intrahepatic blood vessels occur in chronic hepatitis.

The purpose of this paper is to investigate vascular changes in the chronically damaged liver, and also to give some information on the terminal arterial distribution and its relationship to the formation of intralobular collaterals. The liver of the living mouse with chronic carbon-tetrachloride intoxication was observed by a transillumination technique. Simultaneously, the specimens of the liver injected with India ink by a newly modified technique was studied.

Materials and Methods

Materials:
The animals used were seventy commercial male mice. Carbon-tetrachloride in 20 percent olive oil solution was administered subcutaneously at a dose level of 0.1cc per 10g of body weight, repeatedly every 4 days for up to 8 months. After 6 months of the administration, several mice were used as the materials of the recovery stage by stopping further injection of carbon-tetrachloride for two months.

Methods:
Microscopic vital observation by transillumination method
The liver of the living mouse was observed in transmitted light by using a long focus condenser with a transparent animal fixer. The liver was drawn

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out into the physiological saline solution of 37 degrees C. A detailed description of the method employed has been given by Nakata and his coworkers.

Observations of the liver specimens prepared by a new injection methods with India ink
The practical details is as follows: After the mouse had been anaesthetized with urethane and the peritoneal cavity opened, threads had been set up at the site of the entrance and exit of the vessels of the liver and left open to be able to ligate the vessels at any moment. Then a needle was inserted into the heart through the diaphragm, and a small amount of India ink was injected. Two or three seconds later, the threads was tightened all at once. Up to this time the liver had been kept uninterruptedly in its normal circulation. As soon as all the necessary ligatures were tightened the liver was removed from the animal and was bathed in a fixative solution. About 100μ thick frozen sections were prepared subsequently and examined. In the specimens, form and width of the liver vessels has been kept as it was during life, even in the sinusoids.

RESULTS
a) Normal mice
The pattern of the sinusoidal network was fine and orderly on microscopic vital observation (abbreviated as MVO in the following sentences) of the liver. The central and interlobular veins were fairly straight, regularly and

Fig. 1, 2, and 3. Microphotographs of the mouse liver by microscopic vital observation with transmitted light.

Fig. 1. Normal mouse liver. Arrows indicate the direction of blood stream from the interlobular vein (IV) to the central vein (C). Sinusoidal network is fine and orderly. (×100)

Fig. 2. Group 1 (Mouse treated with carbon-tetrachloride (CCl₄) for 3 to 5 weeks). Sinusoidal network seems rough. Arrows indicate enlarged sinusoids. (×100)
not stunted (Fig. 1). Observation by a dissecting microscope failed to show any dilated vessels on the liver surface.

b) Chronic intoxication with carbon-tetrachloride

Each mouse treated for same period not always showed the same change in liver injury, however, it seemed appropriate to divide the animals into five groups as follows.

Group 1: Mice treated with carbon-tetrachloride for 3 to 5 weeks

A fine network of sinusoids changed rough and became irregular (Fig. 2). In the central zone the sinusoids found to be narrowed or obstructed, while in the periphery of the lobule they were dilated showing rather increased blood flow. Enormously enlarged sinusoids were occasionally observed. Central veins became slightly dilated and the blood flow flourished. To the contrary, interlobular veins became slender to some degree. The edge of the liver was slightly undulated, subsiding at the central zone. Histological examination of the sections revealed hepatic-cell degeneration, spotty necrosis predominantly in the centrolobular zone, and also regenerative foci of the cells.

Group 2: Mice treated with carbon-tetrachloride for 2 months

The number of the sinusoids having active blood flow diminished and became less than two or three within the lobule. The diameter of such sinusoids was approximately two times as large as the normals, and all the other sinu-

![Fig. 3. Group 2 (Mouse treated with CCl₄ for 2 months). Arrow indicates more enlarged sinusoids than that of Group 1. (×200)](image)

![Fig. 4. Group 2. Massive necrosis in the central zone. Hematoxolin and eosin stain. (×100)](image)
soids were scarcely recognized (Fig. 3). The enlarged sinusoids were regarded as an early form of intralobular porto-hepatic shunt. Central veins became more widened, while interlobular ones more narrowed and scarcely visible. The widening of drainage vessels was observed despite remarkable narrowing of the interlobular vessels. The fact was suggestive of intervention of the increased arterial flow. The liver parenchym became extremely turbid in this stage. Liver surface appeared whitish and was fine granular. Observation of the tissue sections showed massive necrosis in the central zone, but neither conspicuous infiltration of round cells nor fibrosis (Fig. 4).

With the intention of confirming the manner of the minute circulation of the arterial blood in this stage, India ink was injected into the liver of the living mouse by aforementioned technique. It was noted that by such a method India ink firstly penetrated the lobule only through the hepatic arterial system, then, all the blood flow within the liver is stopped immediately before excessive ink reach there from the portal venous system. The examination of thick frozen sections revealed the fol-

Fig. 5. 6. and 7. The liver injected with India ink through the hepatic artery during life. Group 2 (Mouse treated with CCl₄ for 2 months). Thick frozen section (100μ) stained lightly by hematoxylin and eosin. (×200) (AN: arterial nets, C: central vein, D: bile duct, HA: hepatic artery, IV: interlobular vein, P: portal vein).

Fig. 5. Granules of India ink are found only in the hepatic artery (HA) and arterial nets (AN) around interlobular vein (IV).

Fig. 6. Granules of India ink are found only in the arterial nets (AN) and the enlarged sinusoidal canal (arrow). In all the other part of the lobule, there are no granules of India ink.
ollowing results. India ink was found only in
the hepatic artery as shown in Figure 5. In
the lobules, India ink failed to penetrate the
great part of the sinusoids, except for some
enlarged ones being clearly distinguished from
all the others (arrows in Figs. 6 & 7). No cor-
puscles of India ink were found around the bile
duct. Such enlarged sinusoids was thought to
be consistent with the intralobular shunt ob-
served previously on MVO.

Group 3: *Mice treated with carbon-tetrachloride for 3 months*

In MVO, almost all the sinusoids revealed
the cessation of blood flow, but a few slender
and slightly distorted sinusoids having vigoro
ous blood stream was able to discriminate lu-
cidly from turbid parenchym (Fig. 8). The
latter sinusoids were considered to be cor-
responding to the aforementioned ones which had
conversely been enlarged in the previous stage
(Group 2). The central vein became also tortu-
os and slender. On the surface of the liver, a
remarkable dilatation of the small branches of
the vessels, notably in the hepatic veins, was
observed on microscopic examination with in-
cident light. As shown in Figure 9, the dilated
division was not terminal but somewhat proximal,
and the edge of the liver was markedly
undulated. Histological examination disclosed
that there were much more extensive necrosis
and infiltration of round cells within the lobule
(Fig. 10).

![Fig. 7. Granules of India ink are observed around the interlobular
vein (arrow), while not observed around the bile duct (D).]

![Fig. 8. Group 3 (Mouse treated with CCl₄ for 3 months). Microphotographs of the mouse liver by microscopic vital observation. A few slender sinusoidal canals are seen in the turbid parenchym. Interlobular veins are not visible. (C: central vein)]
Group 4: *Mice treated with carbon-tetrachloride for 4 to 8 months*

An arrangement of the both central and interlobular veins was erratic and disorderly in MVO (Fig. 11). While the central veins were extremely thin and stunted, in more proximal portions of the venous system there was a striking expansion more than in Group 3. These findings were also confirmed by means of microscopic observation of the liver surface in incident light (Fig. 12). Concerning the minute connection between small branches of the portal and hepatic veins observed previously, it was impossible to detect such communications in the transilluminated liver of the living mouse in this stage. In histological findings (Fig. 13), necrotic cells had been disappeared gradually, and were replaced with connective tissue. The disarrangement of the hepatic vascular tributaries, and subsequent disorderliness of the lobular architecture was observed. These features, however, were not considered really cirrhotic.

Group 5: *Mice in regression (After the treatment of carbon-tetrachloride of 6 months' duration)*

In MVO, sinusoidal network became again visible, being rather slightly irregular in contrast with normal, and central veins regained their thickness to a certain degree. Communications between interlobular and central veins were observed in some lobules, and an appear-

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**Fig. 9.** Group 3. A remarkable dilatation of the hepatic vein branches (H) is observed on the surface of the liver with incident light. The edge of the liver is markedly undulated.

**Fig. 10.** Group 3. There are extensive necrosis of hepatic-cells and infiltration of round cells within the lobule. Hematoxylin and eosin stain. (×100)
Fig. 11. Group 4. (Mouse treated with CCl₄ for 4 to 8 months). A microphotograph of the mouse liver by microscopic vital observation. Central veins (C) are thin and stunted, while the sublobular vein (SV) is extremely dilated. Interlobular veins are not visible.

Fig. 12. Group 4. On the liver surface, there is more remarkable dilatation of the hepatic vein branches (H) than those of Group 3 (cf. Fig. 9).

Fig. 13. Group 4. There are disarrangement of the hepatic vascular tributaries, distortion of the lobular architecture and an increase in connective tissue. Azan stain. (×100)

ance of these shunts was characterized by their straightness (arrows in Fig. 14). Microscopic observation of sections revealed disorderliness of hepatic-cell plates, a remarkable regeneration of liver cells without nodule formation, and fibrosis to a minor degree (Fig. 15). In some places, rather straight minute canals were recognized, arising from the portal space toward the central area (Fig. 15). Such canaliculi were considered to be consistent with the canaliculi observed biomicroscopically. In serial sections, the similar anastomoses between portal and hepatic vein branches were also observed as shown in Figure 16.

Figure 17 is a diagrammatic illustration to summarize the results of this study.

**Fig. 14.** Group 5 (Mouse in regression). Microphotograph of the mouse liver by microscopic vital observation. There observed a minute canal (arrow) across the liver lobule from the portal area (PA) to the central vein (C). Interlobular vein is scarcely visible.

**Fig. 15.** Group 5. Minute canal (arrow) starts from the portal toward the central area. Hematoxylin and eosin stain. (×100)

**Discussion**

The new method used in this study reserves some special consideration. Various technique of injection of a colored material into the hepatic vascular tree have been attempted by many investigators, and in almost all instances, ink or other material has been injected into dead livers, even in most recent studies. It is clear that postmortem injection is inadequate to investigate the minute arterial blood flow of the liver, as Elias has also described that “in dead livers ink or other injection masses will flow in any direction, if valves are absent.” He has attempted the injection in living animal by means of CROZONS-
Fig. 16. Group 5. Four microphotographs selected from the serial sections. A minute canal branches off the portal vein. Gradually, the canal approaches the central vein. (Arrows indicate.) Pricrofuchsii stain. (x 50)

Fig. 17. Diagrammatic illustration of the mechanism of intralobular shunt-formation.
(1) Normal mouse liver. Sinusoidal network is fine and orderly. Arterial blood empties directly into some sinusoids through the intralobular arterioles (AO). These sinusoids are arterial in nature (AS).
(II) Early stage in liver injury (Group 1). There are slight disturbance of the intralobular circulation. In such a condition, arterial sinusoids keep rather flourishing blood stream (arrows).
(III) Massive necrosis in central area (Group 2). Almost all the sinusoids revealed the cessation of blood stream. Only arterial sinusoids (AS) have vigorous blood flow. Central vein is dilated, while interlobular vein is narrowed.
(IV) Necrosis covers nearly whole the lobule (Group 3). Arterial sinusoids become slender and distorted. However, they keep vigorous blood flow. Central vein become tortuous and slender. Sublobular vein is markedly dilated.
(V) Precirrhotic stage (Group 4). The central and interlobular vein become extremely thin and stunted. In proximal portion of them, there are striking expansion. Arterial sinusoids in previous stage is now found to exist as communication between portal and central canals.
(VI) Regression (Group 5). Vascularized canal (arrow) in a regenerated lobule is remnant of the intralobular porto-hepatic shunt.

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Zewsky's technique. In his way, however, injection of ink or a dye into the hepatic artery was performed immediately after ligation of the hepatic and portal veins, therefore, it is still not perfectly physiological. In 1932, Nishimaru and Stegenga described an excellent method, which has been modified recently by Ogawa. Yet slight disturbance of the physiological condition would be expected because of the complicated perfusing procedures. A new technique used in this study is more simplified, and any artificial management is not given on the hepatic circulation, just until the granules of India ink arrive at an expected situation in the liver, at least except for anesthetizing and keeping the peritoneal cavity open, if any.

It has been well established by many investigators that there is an increased arterial inflow and formation of venous anastomoses in cirrhotic liver. The present study demonstrated clearly, in the mouse liver, the mechanism of the development of the intralobular anastomosis between the interlobular veins and the central veins, and existence of terminal arterioles which emptied into the deep portion of the lobule.

It is generally accepted that there are arterioles or arterial capillaries which open to the sinusoids. As to the site of opening, Wakiin and Mann, and Elia maintained that terminal arterioles penetrated deep into the lobules, while Kinsely described that the arterial blood entered either into the inlet venules through arterio-portal communications or directly into the portal sinusoids. Hosokawa and Nobuhara concluded on the basis of studies of human and various mammalian livers that in rat livers arterioles empties deep, while not in human livers. Watanabe reported in rat and cat livers that such ones opened to various places in outer half of the lobule.

In the earlier stage of carbon-tetrachloride intoxication (Group 2), the sinusoids, with the development of intralobular circulatory disturbance, were divided into two groups on their attitudes; one became narrow and was deprived of its blood flow, while the other had not only kept its flow, but also possessed more flourishing blood stream. Similar findings were observed by Murashige and Ono. Such difference is considered not by accident but due to distinction between the two groups in an intensity of the blood stream. Murashige considered it questionable that the sinusoid with stronger stream was arterial in nature. On this subject, an interesting studies have been reported recently by Oka and Kanedaka. The sinusoids were divided into three groups according to the grade of minimum pressure needed to stop the sinusoidal flow at various location on microscopic vital observation, i.e. highest, middle and lowest. After the ligation of the hepatic artery, the sinusoidal flow in the group required the highest pressure before the procedure could be arrested with the middle pressure. In contrast, the other two groups revealed no change with such a ligation. These results obtained by Oka and Kanedaka apparently indicate that every sinusoid has various levels in an intensity of its blood flow and the strongest is arterial in nature.

In Group 3 and 4, the intralobular collaterals were observed clearly. Elia has described as follows: "Most of the anastomoses are remnant of previous sinusoids that dilate and whose walls become thick while the surrounding sinusoids disappear. If massive necrosis of whole lobules precedes the formation of cirrhosis, some of remaining sinusoids may persist as communication between portal and central canals." The microphotograph of an enlarged sinusoids, which was about to become a porto-hepatic shunt, has been shown by him. Suzuki has also reported the similar findings in human and rat livers. The canaliculi, which were observed in this investigation and were shown in Figure 3, 6, and 17-III, were very similar to that described by Elia. It was proved, moreover, that such sinusoids accepted arterial blood from arterial capillary net around the small portal veins. It is clear, based on these findings, that such remaining sinusoids after massive necrosis originate from intralobular arterioles or arterial capillaries.

As to the source of the arterial blood the
present investigation revealed that the intralobular arterioles receive it from arterial capillary network surrounding the small portal vein branches but not from the peribiliary arterial plexus, at least in the mouse liver. The existence of the peribiliary arterial plexus in human and various animals has been elucidated by a number of workers; AUNAP (in the cat)\(^2\), ELIAS (in cats and human)\(^3\), MIYAKE (in human)\(^4\), HOSOKAWA (in human and many kinds of animals)\(^5\), and TAJIRI (in human, dogs and toads)\(^6\). Most of them\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) has pointed out communications between the peribiliary arterial network and the intralobular arterioles. MIYAKE has indicated connections of the arterial capillaries and the arterial net around the hepatic artery. The existence of the arterial plexus within the wall of the bile ducts have been reported by WATANABE\(^13\). He has also stated that there was no connection between such a plexus and the portal vein branches. The manner of these communication remains to be determined.

As shown in Figures 3 and 17-III, an increase in width of the central veins was observed in earlier stage of the intoxication, while there observed rather slight decrease in the intralobular veins. This contradiction is incomprehensive without recognition of direct empty of the arterial blood into the lobule. In the succeeding stages, moderate to marked decreases in the width of the central veins were noted also on the side of hepatic venous system (shown in Figures 11, 17-IV, and 17-V). The change was extended frequently to the sublobular veins. More proximal portions of both portal and hepatic venous branches were shown dilated considerably in these stages (Figures 9 and 12).

**Summary and Conclusions**

Microscopic vital observations on the liver of the mouse treated chronically with carbon tetrachloride are reported, with the following conclusions:

1) A new injection method in living animals with India ink has been contrived, for the purpose of detecting the manner of the minute arterial blood flow.

2) Arterial blood empties directly into the lobules through the intralobular arterioles, which are more superior in an intensity of the blood flow than all other sinusoids and keep their flow under the circulatory disturbance in almost all the sinusoids. Eventually, such arterial sinusoids develop to the intralobular porto-hepatic shunt.

3) The intralobular arterioles are derived from arterial capillary network around the small portal vein branches, at least in the mouse liver.

4) In the chronically damaged liver, even if not cirrhotic, there exist abnormalities in intrahepatic hemodynamics. A decrease in diameter occurs somewhere in the intrahepatic venous systems, and the proximal division of such narrowed portion reveals a striking expansion.

5) With the development of hepatic lesions and the formation of porto-hepatic communications, a decrease in width occurs firstly in the most distal portions and subsequently by degrees in the more proximal divisions of the venous branches.

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