Metabolism of Short Chain Fatty Acid in Rat Liver in Biliary Obstruction

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KOYAMA, K., KASHIMURA, S. YAMAUCHI, H., TAKAGI, Y., OWADA, Y. and SATO, T. Metabolism of Short Chain Fatty Acid in Rat Liver in Biliary Obstruction. Tohoku J. exp. Med., 1975, 117 (4), 335-341 — In an attempt to clarify the hepatic insufficiency in obstructive jaundice, hepatic metabolic rate of n-butyric acid, ketogenesis and CO₂ formation from butyric acid were investigated using liver slices obtained from rats subjected to choledochal ligation or carbon tetrachloride (CCl₄) injection. The hepatic metabolic rate of n-butyric acid was reduced with the prolongation of biliary obstruction, and the reduction was presumed to be caused for the most part by the reduced ketogenesis and in part by the impairment of the citric acid cycle (TCA-cycle). In 3~4 weeks after biliary obstruction, the metabolic rate reduced to a level comparable to liver necrosis produced by CCl₄. This reduction of the metabolic rate of n-butyric acid is postulated as one of the pathogenic factors for fatal liver insufficiency in many cases of obstructive jaundice. While the reduction is mild and mobile in the early stage of jaundice, it may be of significance for preventing fatal liver insufficiency to relief the obstruction as early as possible. —— obstructive jaundice; short chain fatty acid; hepatic coma; ketogenesis

While many patients with obstructive jaundice have died of hepatic coma, the real pathophysiological entity of the liver impairment still remains obscure. It is very difficult, therefore, to elucidate the relationship between obstructive jaundice and liver insufficiency. In the present study, the capacity of the liver to metabolize short chain fatty acids (abbreviated as SCFA) that are regarded as one of the important pathogenic factors for hepatic coma was examined, using slices of the liver obtained from rats with obstructive jaundice. The rat liver with CCl₄ poisoning was also studied as an example of serious liver insufficiency with the aim to elucidate the liver impairment in obstructive jaundice.

MATERIALS AND METHODS

Preparation of experimental animals. Obstructive jaundice was induced in Wistar rats weighing about 200 g by ligation and division of the common bile duct. These animals were divided into five groups consisting of 4 to 5 rats each and were sacrificed at 1, 2, 3, 4 and 6 weeks after biliary obstruction (abbreviated as 1, 2, 3, 4 and 6 week groups, respectively) and the liver specimens and blood were obtained for histological and
biochemical analyses. CCl₄ poisoned rats were prepared by intraperitoneal injection of 20% CCl₄ in olive oil at a dose of 1 ml/100 g body weight and were sacrificed 48 hr after CCl₄. The livers were washed with physiological saline at 0°C, and sliced in pieces of 0.3 to 0.5 mm in thickness in a cold room at 4°C.

**Incubation.** The liver slices weighing about 200 mg were incubated in a Erlenmeyer flask with 3 ml of medium (Eagle MEM culture medium*) containing 10 μmoles of sodium n-butyrate as an SCFA at 37°C for 2 hr under pure oxygen with shaking. After incubation, the flask was chilled promptly to stop the reaction, and the medium was separated and subjected to analysis.

**Determination of butyric acid.** The medium after the incubation was treated as described in Fig. 1 and the butyric acid extracted was dissolved in 0.1 ml of a mixture of formic acid-ether (1:4) containing 0.1% dodecan and determined by gas liquid chromatography (Friedmann 1938; Sasaki 1969). The rate of metabolism of n-butyric acid in the liver was calculated by subtracting the amount of n-butyric acid remaining in the medium after incubation from that (10 μmoles) initially added to the medium. The gas-liquid chromatography was performed as follows: Apparatus, hydrogen ionization detection system with the column made of stainless steel, 3 mm in diameter and 175 cm in length; stationary phase, C-SK 100/200; liquid phase, 9% Tween 80+1%H₃PO₄; column temperature, 120°C; detector temperature, 200°C; carrier gas, N₂.

**Determination of ketone body.** Furfural method as illustrated in Fig. 2 was employed (Lyon and Bloom 1958). The amount of ketone bodies produced in the liver after incubation with butyric acid was determined by subtracting the amount of ketone body endogenously produced without sodium n-butyrate added, from the amount of ketone body obtained in the complete system.

**Determination of ¹⁴CO₂.** 3 ml of the medium containing 10 μmoles of ¹⁴C-sodium n-butyrate (0.03 μCi) and 200 mg of liver slice were placed in the main compartment of a Warburg vessel, 0.2 ml of 20% potassium hydroxide solution in the center well, and 0.2 ml of 50% trichloroacetic acid (TCA) in the side arm. The gas phase was filled with pure oxygen. Incubation was performed at 37°C for 2 hr with shaking. The reaction was terminated by the addition of TCA, and the ¹⁴CO₂ generated was absorbed into potassium

![Fig. 1. Method of extraction of short chain fatty acids (steam distilling method).](image)

* purchased from Nissui Seiyaku Co., Ltd.
hydroxide. The potassium hydroxide solution was transferred into a centrifuge tube. 1 ml of 0.1 M Na₂CO₃ and 3 ml of 0.1 M BaCl₂ were added, then the mixture was centrifuged. The precipitates obtained were washed three times with water, two times with ethanol and once with ether, and were transferred to planchet, dried under an infrared lamp and radioactivity was determined by a gas flow counter.

**RESULTS**

**Histological findings**

The histological findings in the liver in biliary obstruction were reported previously (Koyama et al. 1975) which were summarized as follows: the bile duct proliferation and fibrosis usually occurred and their grades progressed with the duration of biliary obstruction, and after 6 weeks, the histology showed biliary cirrhosis. In the liver in CCl₄ injected rats, a marked necrosis of hepatocytes was seen in centrolobular area.

**Results of liver function tests**

As reported previously (Koyama et al. 1975), values of serum bilirubin, GOT, ALP, etc. were elevated markedly at one week after biliary obstruction, keeping mild elevation thereafter. The rise in serum GPT level was not impressive. In the CCl₄ injected group, values of GOT, GPT and ALP were markedly elevated and their mean values were 2247 (Karmen unit), 821 (Karmen unit) and 66.1 (King-Armstrong unit), respectively. The serum bilirubin level was not increased (0.2 mg/100 ml).
Fig. 3. Rate of butyric acid metabolism in rat liver in biliary obstruction or CCl₄ poisoning. ( ), number of animals. * p<0.05.

Fig. 4. Ketogenesis in rat liver in biliary obstruction or CCl₄ poisoning. open circle, endogenous ketogenesis; solid circle, ketogenesis from butyric acid. ( ), number of animals. * p<0.05, † p<0.01.

Rate of n-butyric acid metabolism

The activities of livers to metabolize butyric acid were shown in Fig. 3; the activity decreased along with the duration of biliary obstruction, and the rate of metabolism of butyric acid was most remarkably reduced in the CCl₄ injected group.

Ketogenesis

Endogenous ketogenesis. The hepatic endogenous ketogenesis decreased with the prolongation of biliary obstruction (Fig. 4), a significant difference was
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**Fig. 5.** $^{14}$CO$_2$ formation from butyric acid. ( ), number of animals.

**TABLE 1.** Comparison of extents of decreases in rate of short chain fatty acid metabolism and in ketogenesis in experimental groups as compared with those in the normal group

<table>
<thead>
<tr>
<th>Duration of biliary obstruction (week)</th>
<th>CCl$_4$</th>
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<tr>
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<td>1</td>
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<tr>
<td>Decrease in rate of SCFA metabolism in experimental group (μmole/100 mg liver tissue/2 hr)</td>
<td>0.66</td>
</tr>
<tr>
<td>Decrease in ketogenesis in experimental group (μmole/100 mg liver tissue/2 hr)</td>
<td>0.55</td>
</tr>
<tr>
<td>Ratio of decrease in ketogenesis and decrease in rate of SCFA metabolism (%)</td>
<td>84.6</td>
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observed between the control group and the 2~4 week groups but the difference between the CCl$_4$ injected group and the control group was slight and statistically insignificant.

*Ketogenesis from the butyric acid added.* The ketogenesis from the butyric acid added also decreased along with the duration of biliary obstruction as shown in Fig.4, and the ketogenesis was minimum in the CCl$_4$ injected group. The 2, 3 and 4 week groups and the CCl$_4$ injected group showed statistically significant differences from the control group at a 1% risk. The CCl$_4$ injected group showed statistically significant differences from the 1 and 2 week groups but not from the 3 and 4 week groups.

*Formation of $^{14}$CO$_2$ from the butyric acid added*

The amounts of $^{14}$CO$_2$ formed in various groups were not so significantly different from each other, although the $^{14}$CO$_2$ formation in the CCl$_4$ injected group was considerably smaller as compared with the control group and the biliary
obstruction groups (Fig. 5).

In Table 1 are summarized the differences in the rate of metabolism of butyric acid and of ketogenesis between the groups. Table 1 also includes the ratio of the extents of decrease in ketogenesis and in the rate of metabolism of butyric acid. The results in Table 1 suggest that the decreased rate of metabolism of butyric acid observed in the liver after the prolonged biliary obstruction may be accounted for mostly by the reduced ketogenesis.

**DISCUSSION**

Although many patients with obstructive jaundice died directly of hepatic coma, no remarkable abnormality was seen in the routine laboratory tests. This is probably due to shortage of pathophysiological knowledge on the liver of obstructive jaundice, and our present study was concerned with this respect.

As the possible pathogenic substance responsible for the hepatic insufficiency, the significance of SCFA, as well as ammonia-nitrogen and amines, has been emphasized by Muto and Takahashi (1964). SCFA was presumed to play a leading role in disturbance of consciousness in experimentally induced liver insufficiency in animals which also exhibit other various signs and symptoms such as attack of coma and induction of typically abnormal electroencephalograph (triphasic waves). The reduction in the rate of SCFA metabolism may be taken as an indication of development of hepatic insufficiency (hepatic coma), and the degree of liver impairment may be determined by the SCFA-loading test.

While the reduction of the rate of hepatic SCFA metabolism in serious hepatic impairment had been investigated extensively by Muto and Takahashi (1964) and Walker et al. (1970), there has been no report on the SCFA metabolism in obstructive jaundice. Our present study has demonstrated that the rate of SCFA metabolism was reduced as long as obstructive jaundice persists, and this decrease appeared to be due chiefly to the decreased ketogenesis. However, the possible reduction of the activity of the citric acid (TCA) cycle should also be taken into consideration when biliary obstruction continued for a long period (4 to 6 weeks); the TCA-cycle would be impaired gradually so as to develop insufficient hepatic metabolism of SCFA as encountered in the CCl₄ injected group. This assumption seems to be consistent with the observed reduced formation of ¹⁴CO₂ from butyric acid in the 3 and 4 week groups, and the reduction was especially remarkable in the CCl₄ injected group. However, it should be noted that ¹⁴CO₂ formation observed in the present study may not solely be due to the function of the TCA-cycle, and may also be dependent in part upon the following reaction: acetoacetate→acetone+CO₂.

With respect to the reduced ketogenesis in liver impairment, Fischer and Recant (1956) and Recant (1956) pointed out that blood levels of ketone body in the cirrhotics were markedly lowered. In serious liver impairment, however, blood levels of ketone body are modified by the influence of complicating impairment of the kidney function. These authors removed this uncertain factor by using liver
slices and clearly demonstrated remarkable lowering of ketogenesis in rat liver damaged with methionine (Fischer and Recant 1956; Recant 1956). Disturbance in ketogenesis may be claimed to be a phenomenon common not only in obstructive jaundice but also in various kinds of liver impairment. It should be emphasized that lowered ketogenesis and the decreased SCFA metabolism are closely related each other, depicting a pattern of impaired liver function obstructive in jaundice that has not been advocated before. As biliary obturction persists, these disturbances progress, and within 4 to 6 weeks the disturbances reach up to the same levels as in a typical liver impairment caused by CCl₄, suggesting that obstructive jaundice leads to death due to liver insufficiency. On the other hand, these disturbances are relatively mild in the early stage (1 to 2 weeks) of biliary obstruction. These disturbances seem to be flexible and reversible, and when the relief of obstruction is performed in an early stage, these disturbances may be restored to normal, preventing the aggravation to liver insufficiency.

References