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The Effect of Ketone Body Infusion on Nutritional Support for Liver Regeneration

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Summary. **This study reinvestigated the effects of an infusion of a monoacetoacetin-glucose mixture on rat liver regeneration in the early phase after partial hepatectomy. Animals were either fed an oral diet ad libitum or administered total parenteral feeding containing a monoacetoacetin-glucose mixture, a 20% glucose solution, or a 5% glucose solution. Six rats from each treatment group were sacrificed at 12, 24, 36, 48, and 72 h after partial hepatectomy. The liver regeneration ratio was used as a parameter for liver regeneration in addition to the labeling index and mitotic index. Body weight change, liver functions, plasma concentrations of energy substrates, and plasma levels of insulin were also measured. The rats receiving a monoacetoacetin-glucose mixture increased their liver regeneration ratio to the same degree as the rats fed orally. Monoacetoacetin infused rats also increased both the labeling index and mitotic index similarly to the rats fed orally, while the latter achieved higher calorie intake than did the rats given monoacetoacetin. Those rats infused with a 20% glucose solution showed a delay in increasing these indices. Although the stimulatory effect of monoacetoacetin on DNA synthesis was not shown in this study, it was confirmed that, in the early phase after partial hepatectomy, a monoacetoacetin-glucose mixture had a more beneficial effect on liver regeneration than glucose alone.**

INTRODUCTION

Glucose is known as the primary fuel of the normal liver. It has been reported, however, that a hyperosmolar glucose solution has an inhibitory effect on DNA synthesis in the early phase after partial hepatectomy.[1,2] After partial hepatectomy, nutritional support to the patient is essential, but the optimal nutritional support has not yet been determined.

Monoacetoacetin is a synthetic chemical compound,[3,4] which is hydrolyzed to acetoacetate and glycerol. Several studies have shown that monoacetoacetin given as a mixture with glucose is a useful nonprotein energy source when it is contained in total parenteral nutrition of rats.[5-7] Birkhahn et al. reported a stimulatory effect of this mixture on liver regeneration after partial hepatectomy, suggesting the interaction of hyperketosis induced by monoacetoacetin with the induction of DNA synthesis[8] as its possible mechanism. Their result was based on the changes in DNA synthesis and mitosis. However, regenerated liver weight, which has been recognized as an important parameter of liver regeneration,[9] was not tested in their study. They did not present any data concerning changes in body weight during the experimental period.

This study was performed to reinvestigate the effects of infusing a monoacetoacetin-glucose mixture after partial hepatectomy, using the regenerated liver weight as a parameter for liver regeneration in addition to the labeling index and mitotic index. Changes in body weight and liver function were also measured in this study.

MATERIALS AND METHODS

One hundred and twenty-six male Wistar rats (Charles River Japan Inc., Kanagawa, Japan) weighing 200 to 250 g were housed in a limited access area in the Laboratory Animal Facility of Niigata University at least one week prior to the first sugery. The room was well ventilated with a 12 h regulated light-dark cycle, and the rats were allowed free access to laboratory rat chow (Oriental MF, Oriental Co., Tokyo, Japan) and water. All rats were fasted for 24 h prior to their surgical operations which were completed

between 9 and 11 AM. The rats were anesthetized with ketamine hydrochloride (veterinary ketalar 50, Sankyo Co., Tokyo, Japan, 10 mg/100 g body weight). Except for six rats which were used to take blood samples for the control values, all other rats underwent partial hepatectomy by means of the method proposed by Higgins and Anderson.[10] After hepatectomy, a silastic catheter (Silastic Medical Grade Tubing, 0.020″ i.d., 0.037″ o.d., Dow Corning Corp., Midland, MI, U.S.A.) was placed in the right external jugular vein for continuous feeding. The catheter was secured by a chain and connected to a swivel that allowed the rat to move freely.

The animals were subdivided into four different dietary treatment groups. Group A rats (n=30) were fed orally with laboratory rat chow and water ad libitum after the operation. They were also infused with a 0.9% saline solution at a rate of 1.0 mL per h. Incidentally, most of the earlier studies on liver regeneration after partial hepatectomy used oral feeding. This group therefore provided a link between data obtained by intravenous (i.v.) feeding in Groups B-D in this study and the data with oral feeding in earlier studies. Group B rats (n=30) were fed a total parenteral diet that contained 150 g of monoacetoacetin (the monoglycerol ester of acetoacetate, synthesized as described by Birkhahn and Border[3]) and 50 g of glucose per liter of solution to provide the non-protein calories, and other nutrients listed in Table 1. Group C rats (n=30) were fed a total parenteral diet that contained 200 g of glucose per liter to provide the non-protein calories. The energy density of this diet was equivalent to that of Group B. Group D animals (n=30) were fed a total parenteral diet that contained 50 g of glucose per liter to provide the non-protein calories. The glucose concentration of this diet was equivalent to that of Group B. These three groups of rats were infused at a rate of 1 mL per h for the first 6 h after partial hepatectomy. The infusion rate was then increased to 2.1 mL per h. Rats in Groups B and C were given 43.8 kcal in the first 24 h after surgery, and 50.4 kcal per day in the following two days. Rats in Group D were given 17.5 kcal in the first 24 h, and then 20.2 kcal per day. The mean amount of calories taken orally by Group A rats in the first 24 h was 31.2±3.9 kcal (mean±SEM, n=24). That of the second day was 51.5±3.6 kcal (n=12), which was almost equivalent to the calories given to rats in Groups B and C on the second day after surgery (see above). On the third day, Group A rats increased their oral intake to 67.7±2.5 kcal (n=6), which was about 140% of the calories given to rats in Groups B and C.

Six animals from each of the four groups were sacrificed at 12, 24, 36, 48, and 72 h after the operation. Ninety minutes prior to sacrifice, each rat was given an intraperitoneal injection of bromodeoxyuridine (BrdU, Sigma Co., 50 mg/kg body weight) to label hepatocytes in the S phase of mitosis. Under ketamine anesthesia, the arterial blood was obtained by aortic puncture into a heparinized syringe. The remnant liver was then removed immediately and weighed. One piece of the specimen was kept in 10% formalin solution for hematoxylin and eosin staining. Another piece of the sample was fixed with 70% ethanol for BrdU-staining. The fixed liver tissue was embedded in paraffin, sectioned and mounted on glass slides. Deparaffinized sections were stained with hematoxylin-eosin to count the number of cells in mitosis per 1000 hepatocytes. The other section was stained histochemically with anti BrdU antibody[11]

Table 1. Composition of the parenteral diet

Ingredient	Amount
Energy, g/L	
Group B	
Glucose	50
Monoacetoacetin*	150
Group C	
Glucose	200
Group D	
Glucose	50
Amino acids† , g/L	49.99
Total N	7.985
Electrolytes, mEq	
Na^+	66
K^+	39
Cl^-	66
Ca^{2+}	7.6
Mg^{2+}	5
HPO_4^{2-}	10
Vitamin‡ , /L	2.5 mL
Trace elements§ , /L	1 mL

*Monoacetoacetin (Nippon-Yushi Co., Amagasaki, Japan)
† Proteamin 12 (Tanabe Co., Osaka, Japan).
‡ Neolamin-multi v (Nippon-Kayaku Co., Tokyo, Japan). Each 2.5 mL contains (in miligrams, except as noted): ascorbic acid, 50; vitamin A (retinol), 1650IU ergocalciferol, 5 μg; thiamin, 1.5; riboflavin, 2.0; prydoxine • HCl, 2.0; niacinamide, 20; pantothenic acid, 15.0; tocopheryl acetate, 7.5.
§TEN
Each milliliter contains (in milligrams); Zn, 1.3; Cu, 0.3; Mn, 2.2; I, 0.13.

and the number of positively-stained nuclei per 1000 hepatocytes was counted. The liver regeneration ratio was calculated as follows: the wet weight of the resected liver was divided by its estimated initial liver weight, then multiplied by 100. In a preliminary experiment performed by using the same method of partial hepatectomy, both resected and remnant parts of liver were weighed. The weight of the resected part was 68±2% (n=10) of the sum of resected and remnant parts. As the average of the resected liver weight was 68% of the original liver mass in the preliminary experiment, each of the initial liver weight in this study was estimated as follows: resected liver weight divided by 0.68. The estimation was justified because the same lobules of the liver were resected both in the preliminary and this experiments. After a blood gas analysis was done, each blood specimen was centrifuged at 3000 R.P.M. for 10 min at 4°C. The supernatant was used to determine the concentrations of ketone bodies within the same day of sacrifice. The remaining supernatant was kept at −20°C until the concentrations of fatty acids, glucose, and the plasma levels of insulin were measured. Plasma acetoacetate and 3-hydroxybutyrate concentrations were determined enzymatically (Ketorex, Sanwa Chemicals Inc., Nagoya, Japan). The plasma non-esterified fatty acids levels (NEFA) were measured by an enzymatic calorimetric method (NEFA C-test Wako, Wako chemicals Inc., Osaka, Japan) and the plasma glucose levels were measured by glucose oxidase methods (Glucose C-test Wako, Wako chemicals Inc., Osaka, Japan). Plasma insulin levels were measured by radio-immunoassay (double antibody assay; ARC-950, Aroka Co., Tokyo, Japan). Biochemical studies were only performed for the blood samples collected at 72 h after surgery. The parameters were measured automatically (Hitachi-736, Hitachi Co., Tokyo, Japan) as follows: total protein (TP), Biuret's method; albumin (Alb), bromocrezol green method; glutamic pyruvic transaminase (GPT), LDH-UV method; lactate dehydrogenase (LDH), UV method; total bilirubin (TB), azobilirubin method; blood urea nitrogen (BUN), urease UV method; creatinine (Cre), Jaffe's method. Control values were determined in normal rats (n=6) not subjected to operation after a 24-h fast.

The data were evaluated for statistical significances using a one-way analysis of variance (ANOVA). Scheffe's method was used to confirm statistical significances when differences were found to be significant by ANOVA. For comparison with control values after a 24-h fast, a paired t test was used. The level of significance was set at 1%.

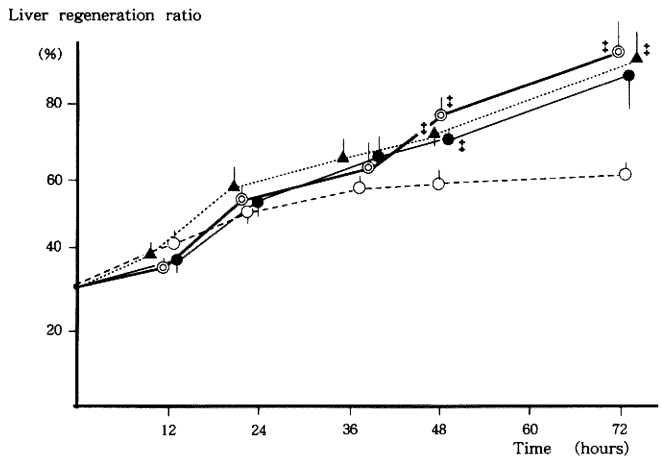

Fig. 1. Liver regeneration ratio (resected liver weight/estimated initial liver weight) in response to different dietary treatment after partial hepatectomy. Values are expressed as mean of 6 animals ± SEM.
‡Significantly different from Group D at comparable time ($p<0.01$)
▲·····▲ Group A (orally fed)
◎——◎ Group B (monoacetoacetin infusion)
●——● Group C (20% glucose infusion)
○·····○ Group D (5% glucose infusion)

RESULTS

The liver regeneration ratio is shown in Fig. 1. In Groups A, B and C, the regeneration ratio increased gradually throughout the experimental period, reaching 92.8%, 93.4%, and 87.2%, respectively, at 72 h after partial hepatectomy. These ratios in these three groups were significantly higher than that in Group D at 48 h after surgery. The regeneration ratio in Group D increased slowly and reached only 62.4% of the original liver weight at 72 h after surgery.

The BrdU labeling indices are shown in Fig. 2. In Groups A, B, and D, the number of labeled cells had already increased at 24 h after partial hepatectomy. The labeling index in Group C was, however, significantly lower at this time point than that in the other three groups. In Group C, the index reached its maximum at 48 h after surgery.

The mitotic indices are shown in Fig. 3. At 24 h after partial hepatectomy, the number of cells in mitosis tended to increase in Groups A, B, and D (significantly increasing in Groups A and D), but the mitotic index in Group C showed a delay to increase until 48 h after surgery. All four groups took their highest value at 48 h after surgery.

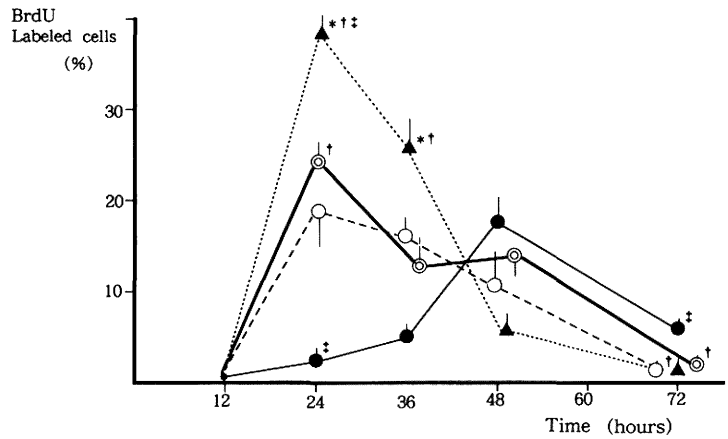

Fig. 2. Labeling index (number of labeled nuclei per 1000 hepatocytes counted) in response to different dietary treatment after partial hepatectomy. Values are expressed as mean of 6 animals ± SEM.
*Significantly different from Group B at comparable time ($p<0.01$)
†Significantly different from Group C at comparable time ($p<0.01$)
‡Significantly different from Group D at comparable time ($p<0.01$)
▲·····▲ Group A (orally fed), ◎——◎ Group B (monoacetoacetin infusion)
●——● Group C (20% glucose infusion), ○·····○ Group D (5% glucose infusion)

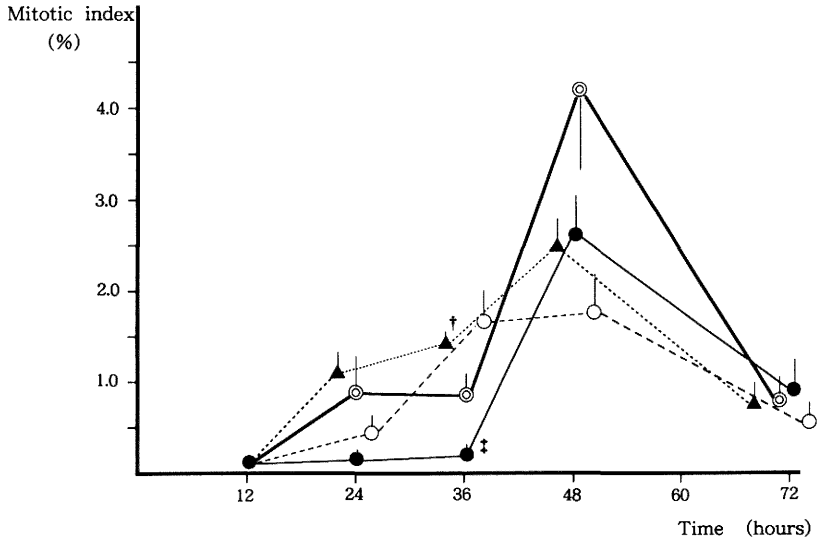

Fig. 3. Mitotic index (number of cells in mitosis per 1000 hepatocytes counted) in response to different dietary treatment after partial hepatectomy. Values are expressed as mean of 6 animals ± SEM.
†Significantly different from Group C at comparable time ($p<0.01$)
‡Significantly different from Group D at comparable time ($p<0.01$)
▲·····▲ Group A (orally fed), ◎——◎ Group B (monoacetoacetin infusion)
●——● Group C (20% glucose infusion), ○·····○ Group D (5% glucose infusion)

Changes in body weight (the ratio of the body weight after partial hepatectomy to that immediately prior to surgery) are shown in Fig. 4. The rats in Group D lost weight during the experimental period. On the other hand, rats in the other three groups maintained their body weight throughout the experimental period except at 12 h following surgery.

The ketone body concentration is presented in Table 2. Rats in Group C had the lowest concentration of all four groups. Group B rats, which received monoacetoacetin, had significantly higher levels of ketone bodies than those of other groups (except the value of 3-hydroxybutyrate at 36 h of Group D).

The plasma NEFA concentration is presented in Table 3. Compared with the 24-h fasting value of 0.774 ± 0.061 mEq/L, the NEFA levels decreased in Groups A-C after enteral or parenteral feeding. The NEFA level in Group B was significantly lower than that in Group A at 24, 36 and 48 h. The NEFA level in Group C was significantly lower than that in Groups A and D at 12, 24 and 36 h, respectively.

Table 4 shows the plasma glucose concentration.

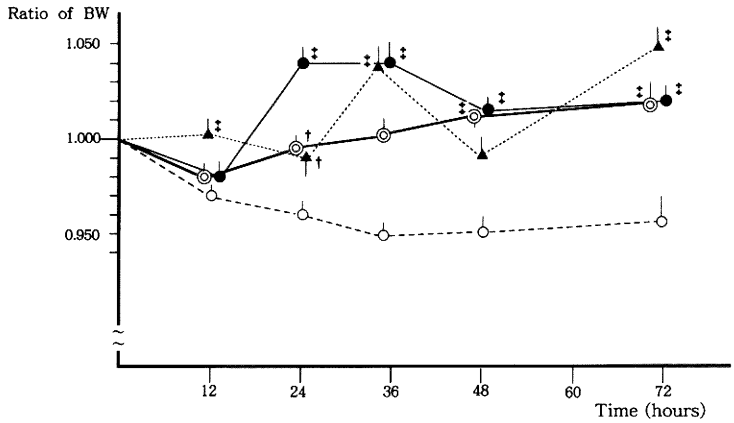

Fig. 4. Body weight change. Each value was the ratio of the postoperative body weight at that to the preoperative weight. Values are expressed as mean of 6 animals ± SEM.
†Significantly different from Group C at comparable time ($p<0.01$)
‡Significantly different from Group D at comparable time ($p<0.01$)
▲·····▲ Group A (orally fed), ◎——◎ Group B (monoacetoacetin infusion)
●——● Group C (20% glucose infusion), ○·····○ Group D (5% glucose infusion)

Table 2. Plasma ketone bodies concentrations (μ mol/L)

Time (hours)	Group A		Group B		Group C		Group D	
	AcAc ‖	3OHBA ¶	AcAc	3OHBA	AcAc	3OHBA	AcAc	3OHBA
12	114.9±39.4*†	267.9±99.3*	272.5±27.5‡§	358.3±77.1*‡	48.6±4.9*	46.8±5.4*	85.6±12.7*	204.1±31.2*
24	70.2±15.6†	133.0±48.4†	339.4±15.1‡§	456.8±56.3‡§	35.9±1.7	35.2±4.3	65.1±2.2	56.1±4.0
36	62.5±8.5†	55.3±10.9†	190.6±34.5‡§	245.6±51.5‡	29.2±4.2	38.3±4.3	100.6±22.4	153.6±25.9
48	76.7±9.1†	62.8±9.3†	335.5±16.5‡§	450.1±59.8‡§	38.7±3.8	37.8±5.2	68.3±9.7	68.1±15.1
72	65.4±6.9†	69.6±6.3†	266.2±48.7‡§	308.9±86.2‡§	44.9±6.8	48.5±4.7	80.3±20.9	101.8±34.0

‖ AcAc; acetoacetate, ¶ 3OHBA; 3-hydroxybutyrate.
The control value of AcAc after a 24-h fast is 299.0 ± 3.62 μ mol/L and that of 3OHBA is 1354.0 ± 113.0 μ mol/L ($n=6$).
Values are expressed as mean of 6 animals ± SEM.
Group A animals were fed orally; Group B animals were infused with monoacetoacetin;
Group C were infused with 20% glucose, and Group D were infused with 5% glucose.
*Significantly different from the control ($p<0.01$)
†Significantly different from Group B at comparable time ($p<0.01$)
‡Significantly different from Group C at comparable time ($p<0.01$)
§Significantly different from Group D at comparable time ($p<0.01$)

Table 3. Concentrations of plasma fatty acids (m Eq/L)

Time (hours)	Group A	Group B	Group C	Group D
12	0.269±0.063*‡	0.111±0.034*§	0.081±0.020*§	0.427±0.052
24	0.229±0.038†‡	0.053±0.015§	0.034±0.003§	0.203±0.061
36	0.274±0.058†‡§	0.041±0.005§	0.061±0.009§	0.500±0.059
48	0.236±0.040†	0.061±0.007	0.091±0.014	0.207±0.068
72	0.171±0.018	0.134±0.037	0.163±0.055	0.165±0.039

The control value after a 24-h fast is 0.774±0.061 mEq/L (n=6).
Values are expressed as mean of 6 animals ± SEM.
Group A-D; refer to Table 2
*Significantly different from the control ($p<0.01$)
†Significantly different from Group B at comparable time ($p<0.01$)
‡Significantly different from Group C at comparable time ($p<0.01$)
‡Significantly different from Group D at comparable time ($p<0.01$)

Table 4. Plasma glucose concentrations (mg/dl)

Time (hours)	Group A	Group B	Group C	Group D
12	145.5±4.3*	113.3±7.8	127.0±17.2	119.1±3.6
24	114.8±7.4	119.9±6.0	147.5±19.3	108.7±4.1
36	141.6±10.8	105.2±7.1‡	166.4±14.1§	116.2±7.0
48	127.4±13.3	120.6±5.4	128.9±8.9	115.8±5.2
72	136.3±10.0	122.7±5.7	132.6±8.7	112.5±7.7

The control value after a 24-h fast is 116.3±4.9 mg/dl (n=6).
Values are expressed as mean of 6 animals ± SEM.
Group A animals were fed orally; Group B animals were infused with monoacetoacetin; Group C were infused with 20% glucose, and Group D were infused with 5% glucose.
*Significantly different from the control ($p<0.01$)
‡Significantly different from Group C at comparable time ($p<0.01$)
§Significantly different from Group D at comparable time ($p<0.01$)

Although the level of plasma glucose was relatively higher in Group C, which was given a high dose of glucose, a significant difference between Group C, B and D was only seen at 36 h after surgery.

Plasma insulin levels are shown in Table 5. After partial hepatectomy, all four groups increased their values, compared with the control value. Group C rats showed the highest insulin level of all four dietary groups with a significant difference at 24 h after surgery. On the other hand, rats in Group D showed a lower insulin level than that in Groups A and C at 72 h after surgery.

The BUN in Group D was 21.0±0.7 mg/dl at 72 h after surgery, which was significantly higher than that in any other group. The values in Groups A, B, and C were 15.1±0.5, 15.1±0.4, and 17.0±1.3, respectively. No remarkable changes were found in other

Table 5. Plasma insulin levels (IU/ml)

Time (hours)	Group A	Group B	Group C	Group D
12	39.8±3.1*	31.5±3.1*	40.0±3.8*	28.8±2.0*
24	31,2±2.0‡	40.3±2.6‡	60.6±6.1§	25.8±1.6
72	26.5±4.1§	23.4±0.8	30.1±2.0§	16.3±2.0

The control value after a 24 h fast is 15.4±1.6 IU/ml (n=6).
Values are expressed as mean of 6 animals ± SEM.
Group A-D; refer to Table 2
*Significantly different from the control ($p<0.01$)
‡Significantly different from Group C at the comparable time ($p<0.01$)
§Significantly different from Group D at the comparable time ($p<0.01$)

blood chemistry including the plasma creatinine level. As for the blood gas analysis, no remarkable acidosis was observed even in Group B rats which infused ketone bodies.

DISCUSSION

Both hyperplasia and hypertrophy of the remnant liver cells occur in the process of liver regeneration after partial hepatectomy. The weight of regenerated liver,[9] labeling index,[12,13] and mitotic index[14] have been used as parameters for liver regeneration. The recovery curves of normalized weights of livers in Groups B and C were almost identical to that in Group A (Fig. 1), although Group A rats took more calories that Groups B and C rats. The liver weight of Group B rats 72 h after surgery was 92.8% of the estimated initial liver weight, which is in accord with the results of earlier studies.[10,15] The liver regeneration ratio of Group D was significantly lower than those of other three groups at 48 h after sugrery. This was probably due to the low calorie intake, as Group D rats received less than half of the calorie given to rats in other groups. In view of the weight of the regenerated livers, infusion of the monoacetoacetin-glucose mixture as well as 20% glucose infusion showed a beneficial effect on liver regeneration.

The labeling index, which reaches the highest value between 20 and 30 h after partial hepatectomy,[12,13,16] increased in Group B as early as in Group A (Fig. 2). DNA synthesis started to increase in Group B much earlier than in Group C even though both groups were provided with the same amount of calories through the experimental period. A similar tendency was found in the time course of changes in the mitotic index of Groups B and C (Fig. 3), although the difference was not statistically significant. Combining the results on liver regeneration ratio and those and those on labeling index, it is suggested that a monoacetoacetin-glucose mixture is a better energy source to facilitate liver regeneration than glucose given intravenously as the sole non-protein source of energy.

An exogenous energy supply should meet the demands not only for liver regeneration but also for the maintenance of normal body functions. Rats in Groups A, B, and C maintained their body weights at the normal control level throughout the experimental period. In Group B, rats gained more weight during the 2-3 days after surgery. Significant weight loss was observed solely in Group D rats, which received a 5% glucose solution (Fig. 4). Group D rats were believed to be short of energy supply. It is probable that the elevation of serum BUN in Group D at 72 h after surgery was the result of an acceleration of catabolism because the rats had no obvious alternation in their creatinine level. These results suggest that a monoacetoacetin-glucose mixture provided enough energy not only to regenerate liver but also to increase body weight following partial hepatectomy in the rats.

There are several possible explanations for monoacetoacetin's facilitatory effect on liver regeneration. (1) Because liver is the central organ in the regulation of carbohydrate and fat metabolism, it supplies energy for the whole body even when the liver itself requires much more energy for regeneration. Monoacetoacetin infusion could share this task of the liver by producing new energy substrates for the whole body. Ketone bodies are useful energy substrates in part because of their excellent penetration and rapid diffusion in peripheral tissue.[17,18] It has been reported that not only can endogenous Ketone bodies preserve body protein but also exogenously infused ketone bodies can conserve protein in fasting patients and post-traumatic patients.[19,20] It is likely that acetoacetate, a derivative of monoacetoacetin, was utilized in preference to either fatty acids or glucose in a critical phase after partial hepatectomy. (2) As Birkhahn et al suggested,[8] ketone bodies may be a better energy source for synthetic process in the hepatocytes than free fatty acids are. Robinson and Williamson have reported that the rapidly dividing hepatocytes of young animals are able to utilize ketone bodies as an energy source.[21] Even in an adult rat, the regenerating liver may regress to a fetal or neonatal form of metabolism and may make use of ketone bodies for an energy source. (3) Recent studies have shown that liver cells prefer long chain fatty acids to glucose as an energy source in the early phase of regeneration following partial hepatectomy.[1,2,22] During the early stage of liver regeneration, NADH, which is produced via enhanced β-oxidation of fatty acids,[23] is accumulated so that the mitochondria of the remnant liver cells become extremely reduced. Because of the relative deficiency of NAD^+, further β-oxidation of fatty acids may be depressed. If acetoacetate which is delivered from monoacetoacetin is converted to hydroxybutyrate in the remnant liver mitochondria (Table 2), this process of conversion may oxidate surplus NADH into NAD^+ coincidentally. The additional oxidation of fatty acids, therefore, would be made possible and could provide further energy supply for hepatocyte regeneration.

Birkhahn et al. have already reported that a monoacetoacetin-glucose mixture shownd a facilitatory

effect on liver regeneration.[8] In their study, DNA synthesis in the regenerating liver increased in rats infused with a monoacetoacetin-glucose more than in rats infused with a 25% glucose solution, in rats infused with a 7% glucose and lipid emulsion solution, and even in rats fed orally. They concluded that hyperketonemia was associated with the induction of DNA synthesis in hepatocytes, although the mechanism was unclear. The persent study, however, did not show the superiority of a monoacetoacetin-glucose mixture to oral feeding in liver regeneration, leaving a difference between the results obtained by Birkhahn et al. and the present ones. The maximum concentration of ketone bodies in the present study was 0.796 mmole/liter, while Birkhahn suggested that ketonemia at an approximate concentration of 1 mmole/liter was effective for the induction. The amount of monoacetoacetin given to Group B rats, therefore, may not be enough to stimulate DNA synthesis in remnant liver cells in this study. However, the amount of the calories taken by the animals can be one of the factors which determines the rate of DNA synthesis.[24] The total of calories fed orally was not presented in Birkhahn's paper. There is, therefore, a possibility that the rats fed orally took fewer calories than the rats infused with monoacetoacetin in their study. Further experiments are needed to clarify the interaction between ketosis and DNA synthesis in hepatocytes after partial hepatectomy.

There is a discrepancy between the labeling index and liver regeneration ratio in Groups C and D, both of which were infused with glucose as a sole source of non-protein calories. Although rats in Group C gained more liver weight than rats in Group D, they showed a delay in DNA synthesis. It is possible that the 20% glucose solution had a depressant effect on DNA synthesis in the remnant liver cells, because it is known that a hyperosmolar glucose solution delays DNA synthesis probably through an inhibitory effect on the fatty acid mobilization.[1,22,25,26] The oversupply of glucose can cause the storage of fatty acids in the remnant liver,[27,28] which may contribute to increased liver weight. An analysis of chemical components of the regenerated liver is necessary to clarify this discrepancy.

It has been reported that mitochondrial ketogenesis is enhanced concomitantly with the enhancement of mitochondrial fatty acid oxidation. This oxidation results in an increase of ATP concentration in the liver after partial hepatectomy.[29] On the other hand, other investigators have failed to demonstrate an increase in the blood ketone body concentrations during post-operative starvation. The result was ascribed to ketone body utilization which overcame its production.[30] In this experiment, the level of plasma ketone bodies in Groups A, C and D was decreased. Rats in Group B had high levels of plasma total ketone bodies. Although ketone bodies were supplied as acetoacetate, the concentration of plasma hydroxybutyrate also increased concomitantly with increased levels of plasma acetoacetate (Table 2). This suggests that acetoacetate was converted to hydroxybutyate.

Plasma NEFA is the main precursor of ketone bodies. NEFA levels in Groups A-C with partial hepatectomy were lower than those of normal fasting rats (Table 3). This result contradicts those of earlier studies that partial hepatectomy resulted in marked and sustained increases in plasma NEFA concentrations.[30-32] Furthermore, rats in Groups B and C had lower NEFA concentrations than those in the two other groups during early periods following partial hepatectomy. Exogenous energy substrates, such as ketone bodies and glucose, inhibit the mobilization of fatty acids by way of insulin secretion. Plasma insulin levels in Group C rats, however, were significantly higher than those in Groups A, B and D at 24 h after hepatectomy. It is, therefore, possible that fatty acid mobilization in Group C rats was suppressed more severely than that of rats in Groups A, B and D. An additional explanation for the low fatty acid concentration in Group B is that fatty acid utilization may be accelerated by a monoacetoacetin infusion through the oxidation of accumulated NADH to NAD^+ in mitochondria. Measurement of the plasma glucagon levels would help toward more complete understanding of the fatty acid mobilization found in this study.

Both hypoglycemia and glucose intolerance have been reported following partial hapatectomy.[1,22,33,34] Neither hyperglycemia nor hypoglycemia, however, was seen in the present study, although only a 5% glucose solution was given to rats in Groups B and D. Plasma glucose levels increased in approximate proportion to the dose of glucose infused in Groups B, C and D during the early phase following partial hepatectomy. Though ketone body infusion is also reported to decrease blood glucose concentration,[35] it is likely that 5% glucose added to monoacetoacetin made it possible to maintain blood glucose concentrations at the normal levels.

The elevation of liver enzyme activities is often observed following partial hepatectomy.[36-38] These changes are related to a combination of several factors including net loss of liver mass and congestion in the remnant liver. Additionally, total parenteral nutrition (TPN) often causes hepatic dysfunc-

tion.[39] Although both partial hepatectomy and TPN were performed in this experiment, the elevation of the activities of liver enzymes was not significant. In this point of view, monoacetoacetin possibly protected the remnant liver cells from harmful overactivity. It is likely that this substance may be utilized in keeping the liver function intact during temporal hypofunction of the liver comparable to partial hepatectomy.

In conclusion, a monoacetoacetin-glucose mixture is a sufficient energy source for liver regeneration, judging from both liver weight gain and increased DNA synthesis. The stimulatory effect of monoacetoacetin on liver regeneration reported by Birkhahn et al., however, was not confirmed in this experiment: i.e., the effect of monoacetoacetin was not superior to oral feeding. Further experiments are necessary to clarify the mechanism of monoacetoacetin in effecting liver regeneration. It is also worth noting that much remains to be explored concerning changes in the metabolic processes evoked by partial hepatectomy, and that better means of nutritional support after partial hepatectomy should be sought.

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