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## Glucose Intolerance after Major Hepatic Resection: Its Mechanism and Clinical Meanings

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### Abstract

Glucose tolerance test is very useful in better understanding the stage of the regenerating process in the remnant liver during the early postoperative period after major hepatic resection. In clinical cases, glucose tolerance test could provide at least some prognostic information concerning operative risk, late survival and the likelihood of early postoperative liver failure in hepatectomized patients. In 70% hepatectomized rabbits, the glucose tolerance patterns after an oral glucose load were characterized by a long-standing increase of blood glucose for more than 180 minutes (a linear pattern) at 24 hours after hepatectomy and by a return toward normal range within 180 minutes (a parabolic pattern) at 96 hours later. At the linear stage, the concentrations of pyruvate and lactate of the remnant liver increased to 192% and 203% respectively of sham-operated controls at 1 hour after an oral glucose load. At 3 hours after glucose load, the concentrations of glucose-6-phosphate, fructose-6-phosphate, pyruvate and lactate increased to 191%, 192%, 174% and 298%, respectively. By contrast, at the parabolic stage, no significant changes after glucose load were observed compared with those of sham-operated controls. The energy charge level of the remnant liver decreased rapidly and reached the lowest level of 0.767 at the linear stage and then returned to near normal level at the parabolic stage. In contrast, mitochondrial phosphorylative activity reached 170% of sham-operated controls at the linear stage and then returned to near normal level at the parabolic stage. Thus, glucose tolerance is closely associated with the changes of the energy charge and mitochondrial phosphorylative activity of the remnant liver. Non-esterified fatty acid levels in the plasma reached the highest level of 0.98 mEq/l at the linear stage compared to 0.46 mEq/l of sham-operated controls. Total ketone body concentrations also reached the highest level of 0.237  $\mu\text{mol/ml}$  at the linear stage compared to 0.133  $\mu\text{mol/ml}$  of sham-operated controls. They then returned to almost normal

Key words: Glucose tolerance, Energy metabolism, Mitochondrial redox state, Major hepatic resection, Glycolytic intermediates.

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levels at the parabolic stage. Blood ketone body ratio decreased rapidly and reached the lowest level of 0.415 compared to 0.726 of sham-operated controls at the linear stage and returned to almost normal levels at the parabolic stage. Portal infusion of (+)-octanoylcarnitine decreased the energy charge level markedly to 0.446 at the linear stage. However, this effect became smaller with the restoration of energy charge, and became insignificant at the parabolic stage. On the contrary, sodium fluoride decreased the energy charge level at the parabolic stage, rather than at the linear stage, although the decreases were all statistically insignificant. At 48 hours after hepatectomy when the energy charge recovered to about 0.80, glucose-6-phosphate dehydrogenase activity and DNA synthesis of the remnant liver enhanced 180% and 13.6 times of the controls, respectively. These results indicate that at the linear stage the cellular energy needed to restore the decreased energy charge of the remnant liver can be efficiently supplied by the enhanced electron transfer associated with excess NADH from  $\beta$ -oxidation rather than glucose oxidation. These findings suggest that the reduced mitochondrial redox potential reflected by the decrease in ketone body ratio, inhibits the entrance of pyruvate into the *Krebs* cycle and result inability for hepatic utilization of glucose, leading to a linear pattern after an oral glucose load.

### Introduction

Although hepatic dysfunction after hepatectomy has been well reported<sup>1,26,28</sup>, there has been no widely accepted methods for classifying or describing the changes in functional reserve of the remnant liver after major hepatic resection. In this regard, we have reported in experimental studies that the decreased hepatic functional reserve was closely associated with decreased energy charge level of the remnant liver<sup>21,33</sup>. It has also been found that a sequence of characteristic changes in glucose tolerance parallel those in mitochondrial phosphorylative activity and energy charge of the remnant liver after major hepatic resection<sup>8,22</sup>. Such changes are presumably a purposeful metabolic reaction tending to maintain the delicate balance of energy metabolism in the regenerating liver. Such considerations make it important to learn about changes in glucose tolerance after major hepatic resection. The glucose tolerance patterns were principally classified into a parabolic pattern characterized by the return toward normal range within 180 minutes and a linear pattern with longstanding increase for more than 180 minutes. It has been suggested that the former is indicative of compensated damage to the liver, while the latter reflects a critically decreased hepatic functional reserve<sup>22</sup>. However the mechanism of parabolic or linear glucose tolerance pattern following major hepatic resection is still not well understood. In the present study, evidence will be presented indicating that in the linear glucose tolerance pattern, the entrance of pyruvate into the *Krebs* cycle is impeded by the reduced mitochondrial redox potential due to the enhanced electron transfer associated with excess NADH from  $\beta$ -oxidation of fatty acids and at the parabolic stage glucose oxidation becomes dominant in the course of the restoration of the mitochondrial redox potential.

## Materials and Methods

In clinical cases, a glucose tolerance test was carried out in four partially hepatectomized patients. After overnight fasting, the glucose tolerance tests were performed in the conventional manner, with blood samples being taken at fasting and subsequently at regular intervals after the patients ingested 50 g of glucose<sup>22</sup>). Blood glucose levels were determined by the o-toluidine method<sup>7</sup>).

In experiments, healthy young rabbits weighing between 1.8 and 2.2 kg were maintained on a diet of Clea-2 (Nippon Haigoshiryo Co. Ltd. Osaka, Japan) and water ad libitum preoperatively for about 2 weeks and then fasted for 15 hours before operation. The rabbits were anesthetized by intravenous injection of 15 mg/kg body weight sodium 5-allyl-5-(1-methylbutyl)-2-thio-barbiturate. For 70% hepatectomy, the left anterior, right anterior and right posterior hepatic lobes were resected. Sham operations were performed by laparotomy and mobilization of the liver. The time courses of the following parameters were studied.

In the oral glucose tolerance test, 4 ml/kg body weight of 50% glucose solution (2 g/kg of body weight of glucose) were administered with an intragastric injection through a catheter after 15 hours of fasting. Blood samples were taken before the administration and at 30, 60, 120, 180 minutes afterwards. Blood glucose levels were determined by the same method in clinical cases.

For the assay of hepatic adenine nucleotides, liver tissue was clamped and frozen in situ with stainless steel tongs precooled in liquid nitrogen. The frozen tissue was removed and immersed in liquid nitrogen. The entire procedure was completed within 10 seconds. The frozen tissue was then powdered by stainless steel mortar and pestle in a liquid nitrogen bath. The powdered tissue was weighed and added to 3 vol. of 5% ice-cold perchloric acid including 1 mmol EDTA solution. The powdered tissue was then homogenized in a glass homogenizer with a mortar-driven pestle and centrifuged at  $10000 \times g$  for 15 minutes at 0 to 4°C. The supernatant was adjusted to pH 5.5 to 6.0 with cold 69% (w/v)  $K_2CO_3$  and recentrifuged at  $10000 \times g$  for 5 minutes at 0 to 4°C. The supernatant was used for analyzing adenine nucleotides. ATP was determined enzymatically with hexokinase and glucose-6-phosphate dehydrogenase by the standard spectrophotometric method, reading the absorbance of NADH at 340 nm<sup>12</sup>). ADP and AMP were determined similarly with lactate dehydrogenase, pyruvate kinase and myokinase<sup>9</sup>).

Mitochondria were prepared by methods previously reported elsewhere<sup>23</sup>). Mitochondrial phosphorylative activity was measured at 22°C at pH 7.4 in a medium containing 0.3 M mannitol, 0.01 M KCl, 0.04 M  $MgCl_2$ , 0.01 M Tris HCl buffer, 0.005 M potassium phosphate buffer, 0.2 mM EDTA and 230  $\mu M$  ADP. Glutamate was added at a concentration of 0.4 mM as a substrate<sup>23,24</sup>).

To measure acetoacetate and  $\beta$ -hydroxybutyrate, 3 ml of arterial blood sample was mixed with 6 ml of ice-cold 6% (w/v) perchloric acid solution immediately after the blood was taken and the mixture was centrifuged at  $10000 \times g$  for 5 minutes at 0 to 4°C. The supernatant was

adjusted to pH 5.5 to 6.0 with cold 69% (w/v)  $K_2CO_3$  and recentrifuged. The supernatant was used to determine the ketone body concentrations. Acetoacetate and  $\beta$ -hydroxybutyrate were measured enzymatically<sup>17,31)</sup>.

Serum non-esterified fatty acid (NEFA) level was measured by the method of LAURELL and TIBBLING<sup>14)</sup>.

The effect of (+)-octanoylcarnitine and sodium fluoride on the energy charge level of the remnant liver were studied. In 70% hepatectomized rabbits, a total amount 5  $\mu$ moles of (+)-octanoylcarnitine or 200  $\mu$ moles of sodium fluoride, which had been dissolved in 20 ml saline and neutralized to pH 7.4 with NaOH, was intraportally infused by an infusion pump through catheter for 3 hours at the specified times after hepatectomy. In control rabbits saline was infused instead of (+)-octanoylcarnitine or sodium fluoride solution. The animals were fed post-operatively, but fasted again for 15 hours before the infusion experiment. Before and after the infusion, hepatic adenine nucleotides contents were determined and the energy charge were calculated<sup>19,20)</sup>.

For the assay of glycogen, glycolytic intermediates and lactate, liver tissue was clamped and frozen in situ with stainless steel tongs precooled in liquid nitrogen at 60 and 180 minutes after the administration of glucose. The frozen tissue was then powdered by stainless steel mortar and pestle in liquid nitrogen bath. The powdered tissue was weighed and added to 5 vol. of 6% (w/v) ice-cold perchloric acid. The powdered tissue was then homogenized in a glass homogenizer with a mortar-driven pestle. 0.2 ml of homogenate was incubated with 0.1 ml potassium hydrogen carbonate solution and 2.0 ml amyloglucosidase solution in a stoppered centrifuge tube with shaking at 40°C for 2 hours. Incubation was stopped by addition of 1.0 ml perchloric acid. After centrifugation the supernatant was used for the determination of glucose from glycogen hydrolysate. The greater part of the remaining homogenate was centrifuged at 10000 $\times$ g for 15 minutes at 0 to 4°C. A part of the supernatant was neutralized with solid  $KHCO_3$  and recentrifuged. The sample was used for the determination of glucose blank. Another part of the supernatant was adjusted to about pH 3.5 with 69% (w/v)  $K_2CO_3$  and recentrifuged. The sample was used for the assay of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, glyceraldehyde-3-phosphate and lactate. The last part of the supernatant was adjusted to pH 2.5 to 5.0 with 2 N KOH and recentrifuged. The sample was used for the assay of pyruvate, phosphoenolpyruvate and D-glycerate-2-phosphate. All determination was made enzymatically by the spectrophotometric method reading the absorbance of NADH at 340 nm<sup>5,6,11,13,18)</sup>. The amount of glycogen was expressed as  $\mu$ mol glucosyl units/g liver tissue and the amounts of glycolytic intermediates and lactate were expressed as nmol/mg liver protein.

In order to measure the DNA synthesis, 100  $\mu$ Ci/kg of methyl- $H^3$ -thymidine (specific activity 46 Ci/mmol) was intravenously injected. Liver tissue was obtained 2 hours after the injection of the radioisotope. The extraction of DNA was performed according to the procedures of SCHNEIDER<sup>27)</sup> and 10 ml of scintillant (0.4% 2,5-diphenyloxazol and 0.02% 1,4-bis-2-(5-phenyloxazolyl)-benzene in dioxane) was added to 1 ml of DNA extract. Tritium radioactivity was

counted in a liquid scintillation spectrometer (ISOCAP 300/NUCLEAR CHICAGO) and correction for quenching was made by the channel ratio method. DNA in the assay extract was determined by diphenylamine reaction. The procedure was a modification of the BURTON'S method<sup>4)</sup> in which sulfuric acid was omitted and the diphenylamine content increased to 2 g/100 ml and thus the final concentration of perchloric acid was 0.5 mol/l<sup>10)</sup>. Highly polymerized calf thymus DNA synthesis was analyzed in duplicate.

For the determination of glucose-6-phosphate dehydrogenase activity, immediately after the biopsy of the liver, the tissue was put on a filter paper to remove most of blood and weighed. The tissue and about 0.04 ml/mg wet weight EDTA-physiological saline solution was added to an ice-cold homogenizer and homogenized for 2 minutes in an ice bath and then centrifuged for 20 minutes at about 0°C and 15000 rpm. The supernatant was used for the assay of glucose-6-phosphate dehydrogenase activity. The time between the liver biopsy and the start of the centrifugation was not more than 5 minutes. All procedure was performed at constant time of the day (8:00–10:00 a.m.). Glucose-6-phosphate dehydrogenase activity was determined spectrophotometrically reading the absorbance of NADPH at 340 nm at constant temperature (at about 25°C). The results were expressed U/g protein of supernatant<sup>16)</sup>.

Protein was determined by the method of LOWRY et al.<sup>15)</sup>. Results were expressed as the mean and standard error or standard deviation. All statistical analyses were based on Student's t test.

## Results

In Figure 1, changes in glucose tolerance after partial hepatectomy was shown in hepatectomized patients. A 29-year-old man (case 1) was successfully subjected to 60% hepatectomy because of hepatic trauma. The linear GTT pattern continued for 10 days after hepatectomy and then changed to a parabolic pattern. A 48-year-old man (case 2) was successfully subjected to extended right hepatectomy because of hepatoma. He showed a parabolic pattern as early as 4 days after hepatectomy. A linear GTT pattern did not occur postoperatively. His postoperative course was uneventful. A 59-year-old man with liver cirrhosis (case 3) was successfully subjected to right hepatectomy. The linear GTT pattern continued for 3 weeks after hepatectomy and then changed to a parabolic GTT pattern. A 37-year-old man with liver cirrhosis (case 4) was subjected to extended right hepatectomy because of hepatoma. The GTT pattern was linear for the entire postoperative period and he died of hepatic failure on the 31st postoperative day. (Figure 1). In Figure 2, the blood glucose curve after an oral glucose load showed a linear pattern at 24 and 48 hours after hepatectomy in rabbits and then a parabolic pattern at 96 hours later. (Figure 2). Table 1 and Figure 3 show the changes in glycogen, glycolytic intermediates and lactate concentrations of the remnant liver following an glucose load at 24 and 96 hours after hepatectomy. In Table 1, results were expressed as the mean and standard deviation. The contents of D-fructose-1,6-diphosphate, dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate were too little to measure accurately. At 24 hours after hepatectomy, the concentrations of pyruvate and lactate increased to 192% and 203% of sham-

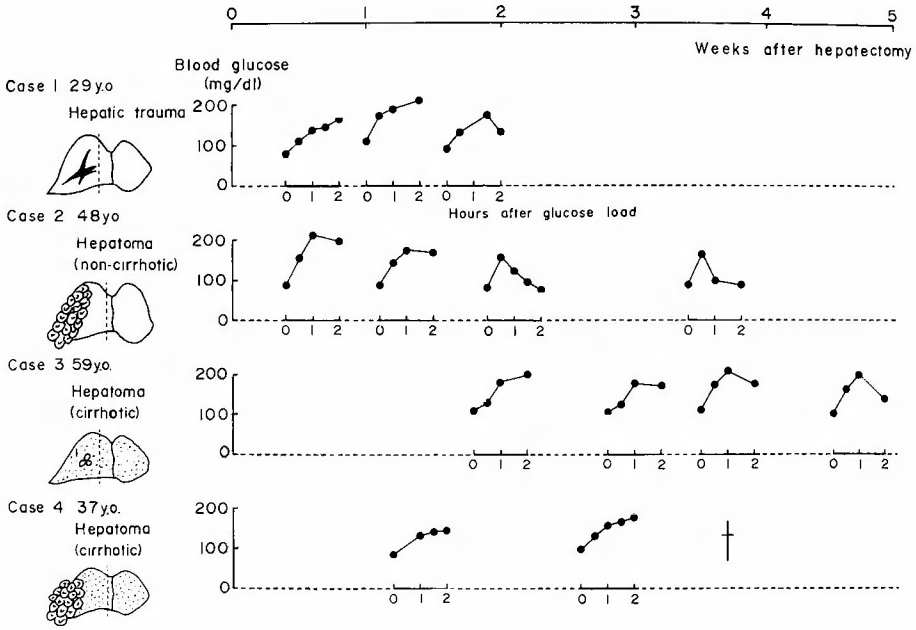


Fig. 1. Changes in glucose tolerance after partial hepatectomy in patients.

operated controls at 1 hour after glucose load, respectively. At 3 hours after glucose load the concentrations of glucose-6-phosphate, fructose-6-phosphate, pyruvate and lactate increased to 191%, 192%, 174% and 298% of sham-operated controls, respectively. By contrast, at 96 hours after hepatectomy no significant changes after glucose load were observed compared with sham-operated controls. (Table 1, Figure 3).

Figure 4 shows the changes in hepatic energy charge, mitochondrial phosphorylative activity, DNA synthesis and glucose-6-phosphate dehydrogenase activity after 70% hepatectomy. The energy charge level of the remnant liver decreased rapidly and reached the lowest level of 0.767 at 24 hours after hepatectomy and then returned to the preoperative level within 7 days. In contrast, mitochondrial phosphorylative activity increased rapidly and reached 170% of the control level at 24 hours after hepatectomy and then returned to preoperative level within 7 days. The incorporation of thymidine into DNA enhanced 13.6 times of the control level 48 hours after hepatectomy when the energy charge recovered to about 0.80. Glucose-6-phosphate dehydro-

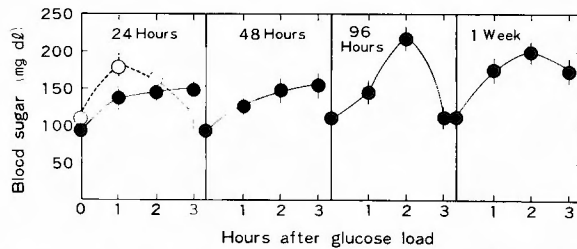


Fig. 2. Changes in oral glucose tolerance test patterns after 70% hepatectomy in rabbits. Open circles indicate normal 15 hours fasted controls.

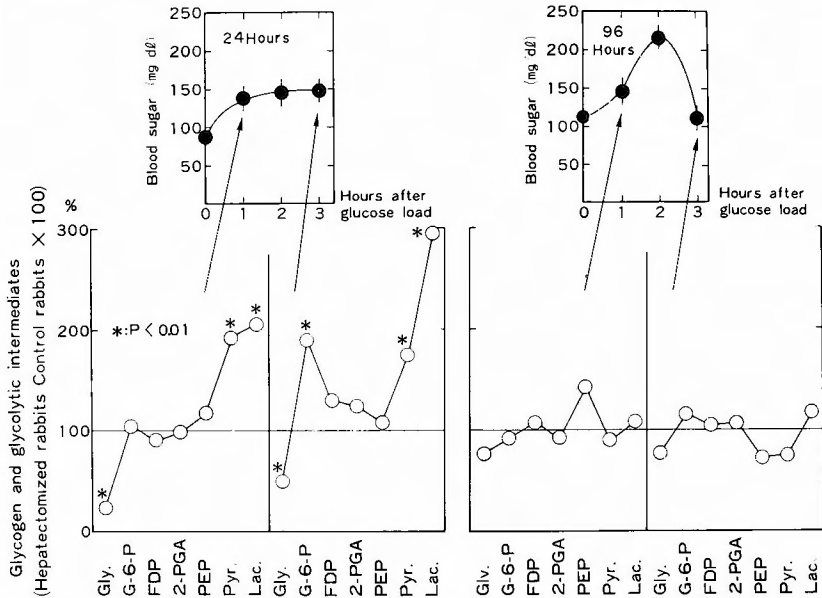
**Table 1.** Changes in glycogen and glycolytic intermediates concentrations after an oral glucose load in 24 and 96 hours hepatectomized rabbits

	24 hours hours after glucose load				96 hours hours after glucose load			
	1		3		1		3	
	sham (n=6)	hepatectomy (n=7)	sham (n=7)	hepatectomy (n=9)	sham (n=4)	hepatectomy (n=8)	sham (n=7)	hepatectomy (n=6)
Glycogen ( $\mu$ mole glucosyl units/g tissue)	16.89 $\pm$ 9.26	4.02 $\pm$ 3.38*	34.51 $\pm$ 6.20	16.91 $\pm$ 6.39*	11.78 $\pm$ 5.34	9.01 $\pm$ 3.65	33.98 $\pm$ 3.95	24.99 $\pm$ 7.60
Glucose-6-phosphate (nmole/mg protein)	0.654 $\pm$ 0.165	0.684 $\pm$ 0.178	1.177 $\pm$ 0.316	2.253 $\pm$ 0.452*	0.499 $\pm$ 0.171	0.493 $\pm$ 0.116	1.257 $\pm$ 0.457	1.444 $\pm$ 0.413
Fructose-6-phosphate (nmole/mg protein)	0.148 $\pm$ 0.037	0.170 $\pm$ 0.043	0.324 $\pm$ 0.065	0.623 $\pm$ 0.116*	0.131 $\pm$ 0.067	0.130 $\pm$ 0.055	0.269 $\pm$ 0.115	0.345 $\pm$ 0.100
Fructosediphosphate (nmole/mg protein)	0.040 $\pm$ 0.019	0.038 $\pm$ 0.009	0.032 $\pm$ 0.007	0.041 $\pm$ 0.008	0.033 $\pm$ 0.008	0.035 $\pm$ 0.007	0.038 $\pm$ 0.009	0.039 $\pm$ 0.012
Triose-3-phosphate (nmole/mg protein)	0.179 $\pm$ 0.025	0.180 $\pm$ 0.028	0.164 $\pm$ 0.016	0.180 $\pm$ 0.032	0.130 $\pm$ 0.031	0.115 $\pm$ 0.026	0.238 $\pm$ 0.057	0.226 $\pm$ 0.042
2-Phosphoglyceric acid (nmole/mg protein)	0.291 $\pm$ 0.078	0.292 $\pm$ 0.077	0.142 $\pm$ 0.021	0.176 $\pm$ 0.035	0.342 $\pm$ 0.020	0.319 $\pm$ 0.088	0.454 $\pm$ 0.084	0.486 $\pm$ 0.090
Phosphoenolpyruvate (nmole/mg protein)	0.792 $\pm$ 0.378	0.930 $\pm$ 0.334	0.537 $\pm$ 0.115	0.592 $\pm$ 0.198	0.661 $\pm$ 0.151	0.944 $\pm$ 0.346	0.936 $\pm$ 0.391	0.677 $\pm$ 0.103
Pyruvate (nmole/mg protein)	0.665 $\pm$ 0.142	1.280 $\pm$ 0.430*	0.378 $\pm$ 0.110	0.656 $\pm$ 0.153*	0.784 $\pm$ 0.061	0.713 $\pm$ 0.234	0.701 $\pm$ 0.248	0.525 $\pm$ 0.089
Lactate (nmole/mg protein)	12.51 $\pm$ 3.79	25.38 $\pm$ 5.11*	5.36 $\pm$ 1.74	15.98 $\pm$ 6.27*	16.28 $\pm$ 3.66	16.91 $\pm$ 2.69	20.04 $\pm$ 4.30	24.17 $\pm$ 5.92

Values in parentheses indicate number of animals

\* Difference significant:  $p < 0.01$

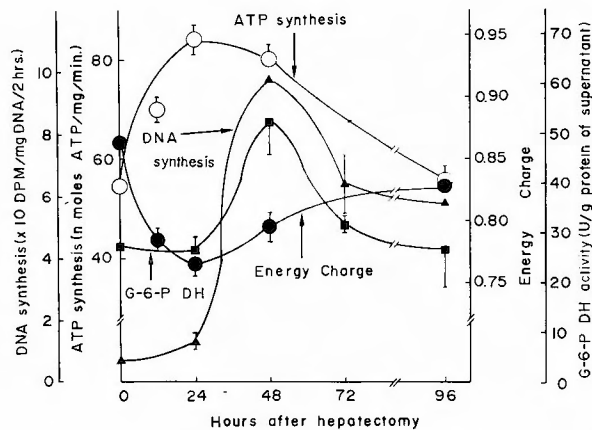




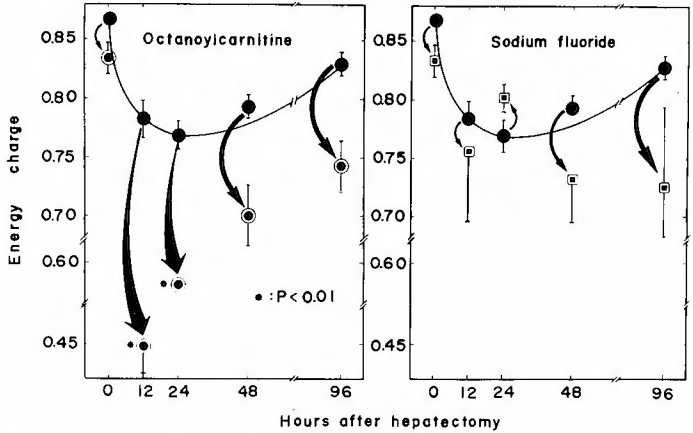
**Fig. 3.** Changes in glycogen and glycolytic intermediates concentrations 1 and 3 hours after an oral glucose load 24 and 96 hours after hepatectomy. Results are shown by percentage of sham-operated controls. Gly.: Glycogen, G-6-P: Glucose-6-phosphate, FDP: Fructose-1,6-diphosphate, 2-PGA: D-glycerate-2-phosphate, Pyr.: Pyruvate, Lac.: Lactate. \* =  $p < 0.01$  compared with sham-operated controls.

genase activity enhanced to 52.9 U/g protein of supernatant compared to 29.8 U/g protein of supernatant of sham-operated controls at 48 hours after hepatectomy. (Figure 4).

The effects of the portal infusion of (+)-octanoylcarnitine and sodium fluoride on the energy charge level of the remnant liver after 70% hepatectomy are shown in Figure 5. Portal infusion of (+)-octanoylcarnitine decreased the energy charge level markedly to 0.446 at 12 hours after



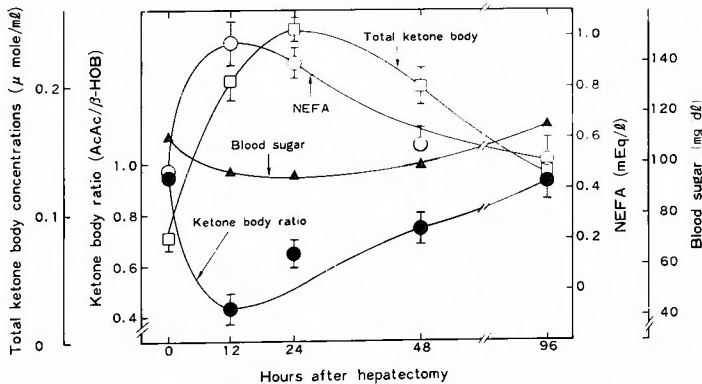
**Fig. 4.** Time course of changes in energy charge level, mitochondrial phosphorylative activity, DNA synthesis and glucose-6-phosphate dehydrogenase activity after 70% hepatectomy in rabbits. Each point represents the mean and standard error of values.



**Fig. 5.** Changes in energy charge level after 70% hepatectomy in rabbits and effects of portal infusion of (+)-octanoylcarnitine or sodium fluoride on the energy charge level of the remnant liver. Statistical analyses were made compared with saline-infused controls. Each point represents the mean and standard error of values.

hepatectomy. However, this effect became smaller and insignificant at 96 hours. On the contrary, sodium fluoride decreased the energy charge level at 96 hours, rather than at 12 and 24 hours, although the decreases were all statistically insignificant. (Figure 5).

Figure 6 shows the changes in NEFA level, total ketone body concentrations (acetoacetate +  $\beta$ -hydroxybutyrate) and ketone body ratio (acetoacetate/ $\beta$ -hydroxybutyrate) in the arterial blood of 70% hepatectomized rabbits. NEFA level increased markedly and reached the highest level of 0.98 mEq/l at 12 hours after hepatectomy. Total ketone body concentrations also increased and reached the highest level of 0.237  $\mu$ mol/ml at 24 hours after hepatectomy. They then returned to almost normal levels at 96 hours. Blood ketone body ratio decreased rapidly and reached the lowest level of 0.415 at 12 hours after hepatectomy before returning to almost normal levels at 96 hours. (Figure 6).



**Fig. 6.** Time course of changes in blood glucose, non-esterified fatty acid level, total ketone body concentrations (acetoacetate +  $\beta$ -hydroxybutyrate) and ketone body ratio (acetoacetate/ $\beta$ -hydroxybutyrate) in arterial blood after 70% hepatectomy in rabbits. Each point represents the mean and standard error of values.

## Discussion

After major hepatic resection the remnant liver cells require much energy for increased metabolic load and following protein and DNA synthesis. So, it can be supposed that after major hepatic resection basic compensatory mechanism appears in energy-generating system. The concept of the energy charge proposed by ATKINSON<sup>2)</sup> as an indicator of cellular energy status is useful for understanding the cells energy flow between energy-utilizing reactions and energy-generating reactions and is normally maintained at a constant level. After major hepatic resection when energy-utilizing reaction increases because of the enormous energy demand, energy-generating reaction also increases. But when energy generation is less than energy utilization and the energy charge decreases markedly, energy utilizing reaction also decreases. After 70% hepatectomy in rabbits, energy charge level of the remnant liver decreased rapidly because of the enormous energy demand. This period appears to be the time when the metabolic overload is maximally imposed upon the remnant liver and the delicate energy balance in the remnant liver is barely maintained by a compensatory enhancement of mitochondrial phosphorylative activity. At this time protein and DNA synthesis does not develop because of their much energy demand and glucose-6-phosphate dehydrogenase is not activated. One of the main metabolic functions of hexose-monophosphate shunt is to supply ribose for DNA synthesis. Glucose-6-phosphate dehydrogenase is thought of rate limiting enzyme of this pathway. This enzyme is induced by high-carbohydrate or lipidless diet after fasting, some hormones such as insulin, thyroxine, estradiol, liver damage and so on, but is repressed by fasting, high-lipid diet, alloxan diabetes and so on. This enzyme induction in damaged liver is not affected by diet or hormones and is non-physiological enzyme induction without RNA synthesis<sup>29)</sup>. Thus, this enzyme induction of the remnant liver at 48 hours after 70% hepatectomy indicates metabolic response as enhancement in hexose-monophosphate shunt and following DNA synthesis. Then liver restoration and regeneration develops. In clinical case 2 the remnant liver already passed through the early-energy-demand stage of the regenerative process at the time of operation. Consequently, removal of the right and middle lobes involved only a relatively small loss in terms of metabolic function. But in clinical case 4 it can be suggested that the delicate energy balance of the remaining liver was barely maintained even during the preoperative period and the mitochondria in the remnant liver could not provide sufficient energy at an early stage of the regenerative process. The result in clinical case 3 indicates that in cirrhotic liver the ability to restore the decreased energy charge is severely suppressed. It is also suggested that hepatectomized patients showing a linear GTT pattern for shorter periods have remarkably greater restorative and regenerative power of the liver after major hepatic resection than those with longer periods. And it seems likely that success of major hepatic resection depends on whether or not the remaining liver has already passed through the early energy-requiring process at the time of hepatectomy. We have reported that glucose tolerance is closely associated with the changes of the energy charge and mitochondrial phosphorylative activity of the remnant liver<sup>8), 22)</sup>. When hepatic energy charge is within normal limits the GTT patterns are parabolic, while linear GTT

patterns develop with the marked decrease of hepatic energy charge. Also, in instances in which the phosphorylative activity of liver mitochondria is within normal limits, parabolic GTT pattern occurs. In instances in which the phosphorylative activity increases or decreases markedly, linear GTT patterns develop. The blood glucose curve after an oral glucose load showed the linear pattern at 24 and 48 hours after 70% hepatectomy in rabbits and then transitioned to the parabolic pattern 96 hours later. At the linear stage, the contents of hepatic pyruvate and lactate increased to 192% and 203% of sham-operated controls, respectively, 1 hour after glucose load. Glucose-6-phosphate, fructose-6-phosphate, pyruvate and lactate increased to 191%, 192%, 174% and 298% of sham-operated controls, respectively, 3 hours after glucose load. These results indicate that the entrance of pyruvate into the *Krebs* cycle is inhibited in the linear glucose tolerance stage. By contrast, at the parabolic stage glycolytic intermediates concentrations did not change significantly compared to those in controls, indicating the restoration of glucose oxidation. These results suggest that the glucose tolerance patterns are closely related to the action of the enzymes of the *Krebs* cycle. The portal infusion of a potent inhibitor of fatty acid oxidation, (+)-octanoylcarnitine, markedly decreased the energy charge level at the linear stage after major hepatectomy. At later infusion, with the restoration of the energy charge, (+)-octanoylcarnitine had less effect and became insignificant at the parabolic stage after the operation. In contrast, the intraportal infusion of an inhibitor of glycolysis, sodium fluoride, did not affect the energy charge at the linear stage after hepatectomy. Afterwards, the infusion of sodium fluoride decreased the energy charge level and enhanced the ketogenesis at 48–96 hours after hepatectomy. These results indicate that ATP synthesis in the remnant liver mitochondria after major hepatectomy is mainly dependent upon the oxidation of fatty acids in the early postoperative critical period, and thereafter is mainly dependent upon the oxidation of glucose in the course of the restoration of decreased energy charge. It seems likely that the energy supply from  $\beta$ -oxidation of fatty acids is more effective than glucose oxidation in restoring the decreased energy charge level of the remnant liver<sup>19,20</sup>. At the linear stage after hepatectomy, the blood ketone body ratio decreased remarkably concomitant with a rise in the blood non-esterified fatty acid concentrations<sup>19,20</sup>. It has been found that the blood ketone body ratio reflects the free NAD/NADH ratio in the mitochondria<sup>21</sup>. With regard to the decrease in blood ketone body ratio, there are two possible explanations: first, an inhibition of the electron transport system, or second, an enhancement of the  $\beta$ -oxidation of fatty acids associated with the production of excess NADH. The first seems to be unlikely, however, because an enhancement of mitochondrial phosphorylative activity was observed 12–24 hours after hepatectomy in rabbits. The second, an enhancement of fatty acid oxidation as indicated by the rise of total ketone body concentrations accompanied by the marked decrease in blood ketone body ratio and rise in NEFA level seems more likely, considering the fact that most of the fatty acid oxidized goes to water soluble products other than ketone bodies, that the liver is the only organ that makes a net contribution of ketone bodies to the blood stream<sup>22</sup> and that the mass of the remnant liver has been reduced to only 30% of the normal liver. It appears that the cellular energy of the remnant liver can be efficiently supplied by the enhanced electron transfer associated with excess NADH

from  $\beta$ -oxidation rather than glucose oxidation in order to restore the decreased hepatic energy charge. The enhancement of  $\beta$ -oxidation of fatty acids results in the production of reducing equivalents (NADH) and acetyl-CoA. Thus, the reduced mitochondrial redox potential inhibits pyruvate dehydrogenase<sup>3, 25),</sup> citrate synthase, isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase. In particular the inhibition of pyruvate dehydrogenase would logically impede the entrance of pyruvate into the KREBS cycle and would result in the inability of the liver to utilize glucose as reflected in the linear pattern after an oral glucose load. By contrast, at the parabolic stage the remnant liver depends upon a greater utilization of glucose in the course of the restoration of energy charge level with its diminished ability to oxidize fatty acids.

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## 和文抄録

広範肝切除後の耐糖能の低下  
—その機序と臨床的意義—入江 龍一, 河野 幸裕, 青山 英久, 中谷 寿男  
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耐糖能検査は広範肝切除後早期における残存肝の再生過程を理解するのに有用である。臨床例においては、耐糖能検査は手術のリスクや肝切除後の生存率及び術後早期の肝不全の発生に関する情報を提供する。70%肝切除後家兎においては、耐糖能曲線は24時間後にみられる180分以上にわたり血糖の上昇を示す linear 型と96時間後にみられる180分以内に下降を示す parabolic 型に特徴づけられる。linear 期においては、残存肝のピルビン酸及び乳酸は糖負荷1時間後には対照群の各々192及び203%に増加した。糖負荷3時間後には、グルコース6リン酸、フルクトース6リン酸、ピルビン酸及び乳酸は各々191, 192, 174及び298%に増加した。これに対し、parabolic 期においては、対照群に比し有意の変化は認めなかった。残存肝エネルギーチャージは急速に低下し linear 期において最低値0.767を示し、次いで parabolic 期にほぼ正常値に復した。これに対し、ミトコンドリアの酸化的リン酸化能は linear 期には対照の170%に達し parabolic 期にはほぼ正常値に復した。このように耐糖能は残存肝のエネルギーチャージ及び酸化的リン酸化能の変化と密接に関係している。血中 NEFA は linear 期に対照の 0.46 mEq/l に比し最高値 0.98 mEq/l に達した。ケトン体総量も linear 期に対照の 0.133  $\mu$  mol/ml に比し最高値

0.237  $\mu$  mol/ml に増加した。これらは parabolic 期にほぼ正常値に復した。血中ケトン体比は急速に低下し、linear 期に対照の0.726に比し最低値0.415に達し parabolic 期にほぼ正常値に復した。linear 期には、オクタノイルカルニチンの門脈内注入によりエネルギーチャージは著明に低下し、0.446に達した。しかしながら、この影響はエネルギーチャージの回復に伴い小さくなり parabolic 期には有意の変化は示さなかった。これに対し、NaF 投与によってはその低下は有意ではないけれども linear 期よりも parabolic 期においてエネルギーチャージは低下した。肝切除後エネルギーチャージが0.80あたりに回復をみせる48時間後に、グルコース6リン酸脱水素酵素活性及びDNA合成能は各々対照の180%及び13.6倍に増加した。これらの結果は linear 期においては残存肝の低下したエネルギーチャージを回復させるのに必要なエネルギーは $\beta$ 酸化よりNADHの産生を伴う電子伝達系の亢進によりグルコース酸化よりも効果的に供給され得ることを示している。これらの所見よりケトン体比に反映されるミトコンドリア内酸化還元比の低下はピルビン酸のTCA cycle への進入を阻害し、肝の糖利用は抑制されその結果糖負荷検査は linear 型を示すことになる。