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Carbohydrate metabolism of rats with biliary obstruction.

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Abstract

Carbohydrate metabolism of rats with obstructive jaundice caused by bile duct ligation was studied by intravenous glucose tolerance test (IVGTT) and by liver perfusion. The altered levels of carbohydrate-metabolizing enzyme were examined in relation to the glucose metabolism of the cholestatic rats. In the IVGTT, the rate of fractional glucose removal was increased with increases in plasma insulin and glucagon and with a decrease in non-esterified fatty acid. In liver perfusion, neither the glucose uptake nor insulin extraction by the whole liver of icteric rats was different from the control. The increased rate of glucose removal in IVGTT may be due to enhanced glucose utilization by peripheral tissues resulting from hypersecretion of insulin. In liver perfusate supplemented with glucose, a decrease in the glucose uptake per unit liver weight was observed in relation to the lowered glucokinase activity. Formation of glycogen from glucose and of glucose from lactate was also impaired, indicating inhibition of the gluconeogenic system or relative hyperfunction of the glycolytic system, which may further contribute to the reduction in glycogen content. These metabolic disorders correlated well with the changes in activities of key carbohydrate-metabolizing enzymes, which showed a characteristic pattern consistent with the loss of differentiated hepatic functions. Uptake of glucose and its conversion to glycogen were reduced in the cholestatic liver in close association with altered activities of some of related enzymes. However, due to increased utilization by the peripheral tissues, the total amount of glucose utilized in the whole rat was not reduced.

KEYWORDS: carbohydrate metabolism, obstructive jaundice, liver perfusion, intravenous glucose tolerance test, glycogen

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Carbohydrate Metabolism of Rats with Biliary Obstruction

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Carbohydrate metabolism of rats with obstructive jaundice caused by bile duct ligation was studied by intravenous glucose tolerance test (IVGTT) and by liver perfusion. The altered levels of carbohydrate-metabolizing enzyme were examined in relation to the glucose metabolism of the cholestatic rats. In the IVGTT, the rate of fractional glucose removal was increased with increases in plasma insulin and glucagon and with a decrease in non-esterified fatty acid. In liver perfusion, neither the glucose uptake nor insulin extraction by the whole liver of icteric rats was different from the control. The increased rate of glucose removal in IVGTT may be due to enhanced glucose utilization by peripheral tissues resulting from hypersecretion of insulin. In liver perfusate supplemented with glucose, a decrease in the glucoce uptake per unit liver weight was observed in relation to the lowered glucokinase activity. Formation of glycogen from glucose and of glucose from lactate was also impaired, indicating inhibition of the gluconeogenic system or relative hyperfunction of the glycolytic system, which may further contribute to the reduction in glycogen content. These metabolic disorders correlated well with the changes in activities of key carbohydrate-metabolizing enzymes, which showed a characteristic pattern consistent with the loss of differentiated hepatic functions. Uptake of glucose and its conversion to glycogen were reduced in the cholestatic liver in close association with altered activities of some of related enzymes. However, due to increased utilization by the peripheral tissues, the total amount of glucose utilized in the whole rat was not reduced.

Key words : carbohydrate metabolism, obstructive jaundice, liver perfusion, intravenous glucose tolerance test, glycogen

The liver plays a central role in keeping the homeostasis of the blood sugar level by regulating the activities of key carbohydrate-metabolizing enzymes of glycolysis and gluconeogenesis. Kurose (1) has demonstrated that the activities of some key carbohydrate-metabolizing enzymes in the liver of rats with biliary obstruction change in a manner similar to those observed in experimentally injured (2), hepatitic or cirrhotic liver (3) in the following mechanism: The activities of glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatse (FBPase) and glucokinase (GK) decrease. These enzymes are all specific to the liver and important for differentiated function of

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the liver. The activities of low-Km hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase type M_2 (PK-M_2) increase. These enzymes are not specific to the liver. The changes in the enzyme activities in parenchymal liver injury appear to be closely related to the decreased glucose tolerance associated with liver injuries. Taketa *et al.* (4) reported correlations between the activities of HK and GK in biopsied liver tissues and the degree of blood sugar elevation in the oral glucose tolerance test in patients with hepatitis and liver cirrhosis. Based on these results, they suggested that decreased GK activities due to hepatic injury are an important cause of impaired glucose tolerance in liver disease. Glucose tolerance in patients with obstructive jaundice has been shown to be decreased (5, 6), but, there have been no reports referring to the relationship between abnormal glucose tolerance and changes in the activity of key carbohydrate-metabolizing enzymes. Impaired glucose tolerance in obstructive jaundice is ascribed to a low insulin response to glucose (5). However, many cases of cancer of the pancreatic head, which is considered to induce hyposecretion of insulin secondary to chronic pancreatitis, are included in those cases, and the influence of obstructive jaundice itself on glucose metabolism has not been fully evaluated.

In experimental studies on rats with biliary obstruction, the tolerance of intravenously administered glucose is reported to be increased (7) and the tolerance of orally administered glucose to be decreased, independent of the insulin status (8). In view of these results, attempts were made to see whether the altered levels of carbohydrate-metabolizing enzymes have any significance in the glucose metabolism of rats with obstructive jaundice. An intravenous glucose tolerance test (IVGTT) and liver perfusion were carried out on rats with bile duct ligation, and the metabolite concentrations and hepatic enzyme activities of the rats were analyzed.

Materials and Methods

Experimental animals. Male Sprague-Dawley rats (Clea Japan Inc., Osaka Japan) kept for more than one week until they attained body weights of 220–280 g were used. The animals were fed on Oriental Laboratory chow MF and water *ad libitum* in cages kept at a temperature of 22–24 °C under a 12 h-lighting regimen.

Bile duct ligation. Rats were anesthetized with an intraperitoneal injection of pentobarbital (5 mg/100 g body weight (B.W.)), and the bile duct was ligated and dissected at a position one third to the hepatic side (9). Control rats underwent only ablation of the hepatoduodenal ligament in the same surgical procedure. The rats were given food *ad libitum* immediately after the operation. They were subjected to experiments on the 7th postoperative day after a 17-h fast. Before sacrifice, rats were anesthetized as for the ligation.

Intravenous glucose tolerance test. The IVGTT was performed as follows: A polyethylene tube of 0.6 mm outer diameter was placed into the inferior vena cava from the femoral vein, and a 50 % glucose solution (1 g glucose/kg B. W.) was injected within 15 sec. Before, and 5, 10, 20, 30, 40 and 50 min after the glucose administration, 0.2 ml of blood was collected from the tube and replaced it each time with the same quantity of physiological saline. Plasma was separated and subjected to glucose and non-esterified fatty acid (NEFA) assays. Laparotomy was performed at 60 min, and approximately 3 ml of blood was collected by direct puncture of the inferior vena cava for determinations of plasma levels of immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) together with glucose and NEFA concentrations. Since a large quantity of blood from each rat was required for these assays, other rats given the same treatment were used for collection of approximately 3 ml of blood for the measurement of IRI and IRG before and 5 and 10 min after the glucose loading. According to the method of Greville (10), the rate of fractional glucose removal (K value) was calculated from plasma glucose levels 10, 20, 30 and 40 min after the glucose loading. Glucose and NEFA concentrations were determined enzymatically (11, 12), and IRI and IRG levels were measured by the two-antibody method (13, 14). A specific antibody OAL-123 (14) was used for the assay of IRG.

Determination of enzyme activities and glycogen contents in the liver after IVGTT. Upon completion of the IVGTT, livers were excised quickly, washed in ice-cold physiological saline and weighed, and then 4

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volumes of 0.154 M KCl-4 mM EDTA (pH 7.5) were added to the livers to prepare homogenates. Part of each liver homogenate was used for the determination of G6Pase activity according to the method of Koide $et\ al.$ (15). The supernatant obtained by centrifugation of the remaining homogenate at 25,000 x g for 60 min was used for determination of GK and HK activities at 37 °C by the method of Viñuela (16), using a model 240 Gilford spectrophotometer. Protein concentrations of the homogenates and supernatants were determined by the method of Lowry $et\ al.$ (17). The specific activities of enzymes were expressed as units (moles of substrate transformed per min under the above assay conditions) per g of protein. Enzyme activities were also expressed as units per g of liver.

Glycogen contents in the liver of fasted rats and of rats after completion of the IVGTT were measured according to the method of Keppler (18).

$Liver\ perfusion.$ $In\ situ$ perfusion of the liver using 22 mM glucose as a glycolytic substrate or 8.4 mM lactate as a gluconeogenic substrate was performed according to the method of Mortimore (19). A perfusate was prepared by suspending bovine erythrocytes in Krebs-Ringer bicarbonate buffer (KRB) containing 3 % bovine albumin to give a hematocrit value of 25 % and adding glycolytic or gluconeogenic substrate to the erythrocyte suspension. Bovine erythrocytes were prepared from bovine arterial blood collected in the acid citrate dextrose (ACD) solution. Red blood cells were separated by centrifugation at 3,000 rpm and 0–5 °C, washed twice in two volumes each of ice-cold physiological saline and KRB. The perfusion medium was equilibrated with a mixed gas of 95 % O_2– 5 % CO_2 at a flow rate of 400 ml/min. Closed-system perfusion was carried out at 37 °C, pH 7.4 and a rate of 8–9 ml perfusate/min. After 20 min of preperfusion with 50 ml of the perfusate, the medium was replaced with 50 ml of fresh perfusate for 60 min of perfusion. Using metabolite concentrations determined in perfusates before and after perfusion, calculations were made of glucose uptake and insulin extraction by the liver when 22 mM glucose and 1 unit/5 g glucose of insulin (Regular Insulin, Novo Chem. Co., Denmark) was added. Lactate uptake and glucose production by the liver was determined when 8.4 mM lactate was added. Since approximately 5 ml of perfusate leaked during the 60 min of perfusion, a correction was made in the calculations. For cases without leakage, a formula of $[C_1-C_2] \times V/t$, where C_1, C_2, V and t represent the initial and final concentrations of the substance concerned, the volume of perfusate and the time interval, respectively, was used. For cases with leakage, a formula of $[C_1-C_2] \times (V_1-V_2)/(\ln V_1 - \ln V_2) \times t$, where V_1 and V_2 represent initial and final volumes of perfusate, respectively, was used, assuming a constant rate of leakage. Portions of livers were excised immediately after the completion of perfusion, frozen in liquid nitrogen within 5 sec, powdered, and stored in liquid nitrogen until subjected to determination of metabolite levels. After frozen powder was placed in a chilled centrifuge tube, 2 volumes of ice-cold 30 % $HClO_4$ were added to it. The homogenates were mixed with 3 volumes of 4 mM EDTA and centrifuged at 0 °C. The supernatants were decanted and neutralized with 5 M K_2CO_3 in ice-cold water. After removal of the precipitate which formed in 15 min at 0 °C, the concentrations of the following metabolites in the final supernatant were determined by enzymatic methods which employed either the oxidation of NADH or the reduction of NAD and NADP (20 – 27): Glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1, 6-bisphosphate (FBP), glyceraldehyde-3-phosphate (GAP), dihydroxyacetone-phosphate (DHAP), phosphoenolpyruvate (PEP), pyruvate, lactate, ATP, ADP and AMP. The hepatic energy charge was defined in terms of actual concentrations as [ATP + 1/2 ADP]/ [ATP + ADP + AMP] (28). For calculation of the ratio of dilution, the volume of 5 M K_2CO_3 required to neutralize $HClO_4$ extracts was determined by dividing the difference in weight between the extracts before and after neutralization by the specific gravity (0 °C, 1.45) of 5 M K_2CO_3, assuming that 0.75 ml of water was contained in 1 g of liver. The lactate concentration in the perfusate was measured according to the procedure of Noll (25) used for the determination of liver metabolites. The ratio of dilution was determined from the specific gravity of the perfusate (1.05) and the water content of the blood (80 %). The hepatic glycogen content after the perfusion with 22 mM glucose and insulin was measured by the method of Keppler (18). As controls to perfusion experiments, levels of liver metabolites of non-perfused fasting rats were measured similarly after excision of the livers under anesthesia, followed by freezing of the obtained samples.

$Chemicals.$ The reagents used as substrates or cofactors were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and purified enzyme preparations from Boehringer (Manheim, Gmbh, West Germany). Other reagents were of the highest commercially-available grade.

$Statistical\ analysis.$ The experimental results were expressed as the mean $\pm$ SD, and significance of

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differences were analyzed by Student's *t* test.

Results

Changes in body weight and wet liver weight following bile duct ligation are shown in Table 1. Although the body weight was not affected by bile duct ligation, wet liver weight and the ratio of liver weight to body weight increased significantly after the treatment. Results of liver function tests are given in Table 2. Significant and marked increases in the serum levels of total bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase as compared with levels in the control group were observed.

Intravenous glucose tolerance test. Changes in plasma levels of glucose and NEFA in the IVGTT are shown in Fig. 1. The icteric group showed a significantly low fasting plasma glucose level (108.1 ± 10.3 mg/dl) as compared with the control group (116.6 ± 10.3 mg/dl). The plasma glucose level reached a peak (426.7 ± 25.5 mg/dl

for the icteric group and 434.7 ± 38.2 mg/dl for the control group) 5 min after glucose administration and decreased exponentially thereafter. Although no significant difference was observed in the plasma glucose level between the two groups up to 20 min after initiation of glucose loading, the icteric group showed significantly low plasma glucose levels after 30 min, recovering to the pre-loading level after 50 min (107.0 ± 15.1 mg/dl). The plasma glucose level 60 min after glucose loading in the control group was still higher (138.2 ± 16.2 mg/dl) than the pre-loading level. The K value in the icteric group was 3.66 ± 0.50 %/min, which was 1.3 times ($p < 0.001$) the K value in the control group (2.88 ± 0.58 %/min), indicating an increased glucose utilization. The NEFA level before glucose administration was significantly lower in the icteric group (52.6 ± 15.7 mEq/L) than in the control group (74.1 ± 19.0 mEq/L). The NEFA level decreased after glucose administration reached nadirs in 30 min in both groups (11.2 ± 2.8 mEq/L in the icteric group and 20.5 ± 9.7 mEq/L in the control group), and increased thereafter. The

Table 1   Effect of bile duct ligation on body and liver weight

| Animals | Number of animals | Body weight (g) | | Liver weight (g) | Liver wt/ Body wt (%) |
		Day 0	Day 7	Day 7	Day 7
Control	15	265 ± 25	266 ± 19	8.5 ± 0.7 ***	3.2 ± 0.2 ***
BDL	20	265 ± 22	254 ± 19	13.1 ± 1.2	5.1 ± 0.3

Values are the mean $\pm$ SD.   BDL, bile duct ligation.   ***, $P < 0.001$.   wt, weight

Table 2   Effect of bile duct ligation on the results of liver function tests

| Animals | Number of animals | Liver function test (Day 7) | | | |
		Bilirubin (mg/dl)	AL-Pase (K.A.U.)	GOT (K.U.)	GPT (K.U.)
Control	15	0.2 ± 0.1 ***	15.8 ± 3.2 ***	66 ± 12 ***	13 ± 4 ***
BDL	20	9.6 ± 1.6	28.4 ± 4.5	565 ± 201	135 ± 58

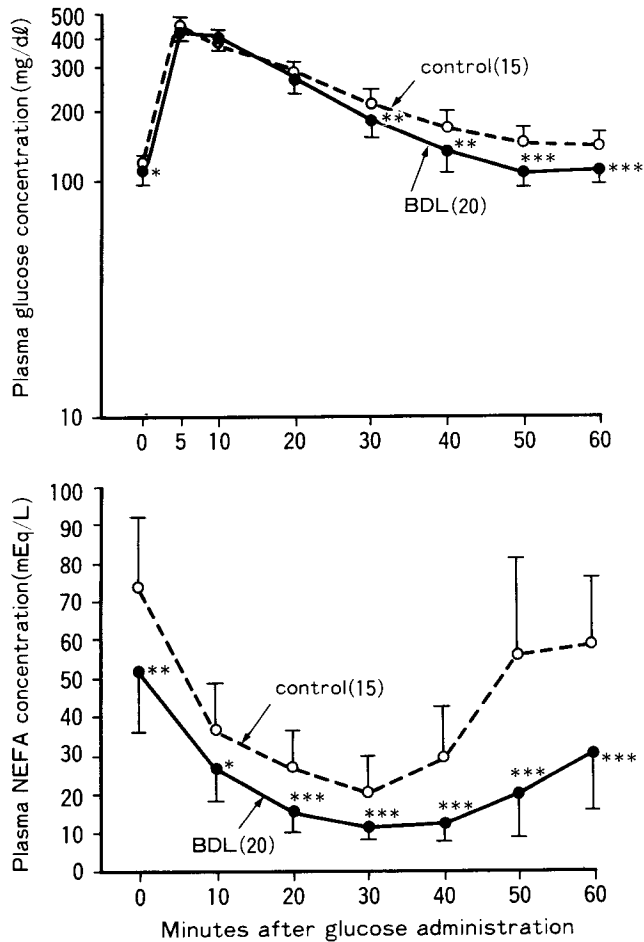Values are the mean $\pm$ SD.   Symbols, see the legend to Table 1.

Fig. 1   Plasma glucose (top panel) and NEFA (bottom panel) concentrations after intravenous glucose (1 g/kg) administration. ●———●, bile duct ligation (BDL) ; ○‐‐‐○, sham-operation. The glucose concentration is plotted on a logarithmic scale. Values are the mean ± SD, with the number of observations in parentheses. ∗, P < 0.05; ∗∗, P < 0.01 ; and ∗∗∗, P < 0.001 for the differences from the control.

NEFA level in the control group recovered to 79 % (58.5 ± 17.9 mEq/L) of the pre-loading level in 60 min, while the NEFA level in the icteric group recovered to only 57 % (30.1 ± 14.7 mEq/L) of the pre-loading level in 60 min.

Plasma levels of IRI after glucose administration are shown in Fig. 2. The IRI levels before and 5, 10 and 60 min after glucose loading were significantly higher in the icteric group than in the control group. Peak levels of IRI were observed in both groups 10 min after glucose administration, the values being 59.7 ± 13.2 μU/ml for the icteric group and 39.5 ± 8.3 μU/ml for the control group. The increase in the IRI level after glucose loading was two times greater in the icteric group than in the control. The IRG of the icteric group (92.9 ± 31.6 pg/ml) before glucose loading was 2 times that of the control group (46.1 ± 12.1 pg/ml). Contrary to the increase in IRI, the IRG level decreased in both groups in 5 to 10 min and recovered to the pre-loading levels within 60 min. The icteric group showed IRG levels significantly higher than those of the control group throughout the experimental period.

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Contents of liver glycogen are shown in Table 3. Liver glycogen was nearly completely depleted as a results of 17-h of fasting in both groups. Although the icteric group had a slightly lower level, there was no significant difference between the two groups. Liver glycogen content increased within 60 min of glucose loading in both groups, although less glycogen accumulated in the whole liver in the icteric group (33 % of the control level). The amounts of liver glycogen deposited

after the glucose load were 4.4 ± 3.5 % and 13.2 ± 7.6 % of the amount of glucose administered to rats with bile duct ligation and sham operation, respectively.

Table 4 shows the activities of key carbohydrate-metabolizing enzymes in the liver of bile duct-ligated and control rats. GK and G6Pase, which are considered liver-specific and important for the expression of differentiated liver function, decreased markedly in activity per unit liver weight to 30 % and 43 % of the control, respectively, following bile duct ligation, while HK, an ubiquitous enzyme showed an increase in activity 2.8 times the level of control liver. Although the sum of GK and HK activities per unit liver weight decreased to 87 % of the control value, the sum of GK and HK increased slightly (1.4 times that of the control value) in terms of the activity of the whole liver as a result of the liver enlargement caused by the bile duct ligation. The (GK + HK)/G6Pase ratio in the icteric group was also approximately twice as high as the ratio in the control group, indicating a potentially higher phosphorylating capacity of the liver after bile duct ligation.

In the control group, significant correlations were observed between the liver glycogen content and the (GK + HK)/G6Pase ratio and K value, while in the icteric group, no significant correlation was observed between the liver glycogen content and any of the other parameters (Table 5). In summarizing the above results of *in vivo* experiments, 1-week bile duct ligation caused an increase in the rate of fractional glucose removal with concomitant elevations in plasma insulin and glucagon levels and a marked decrease in liver glycogen content.

Liver perfusion. In order to study the direct effect of bile duct ligation on the metabolic disorders of the liver, *in vitro* experiments were carried out using perfused livers.

Hepatic glucose uptake, glycogen content and insulin extraction in liver perfusate are presented in Table 6. Glucose uptake, glycogen deposition and insulin extraction per unit liver weight in the
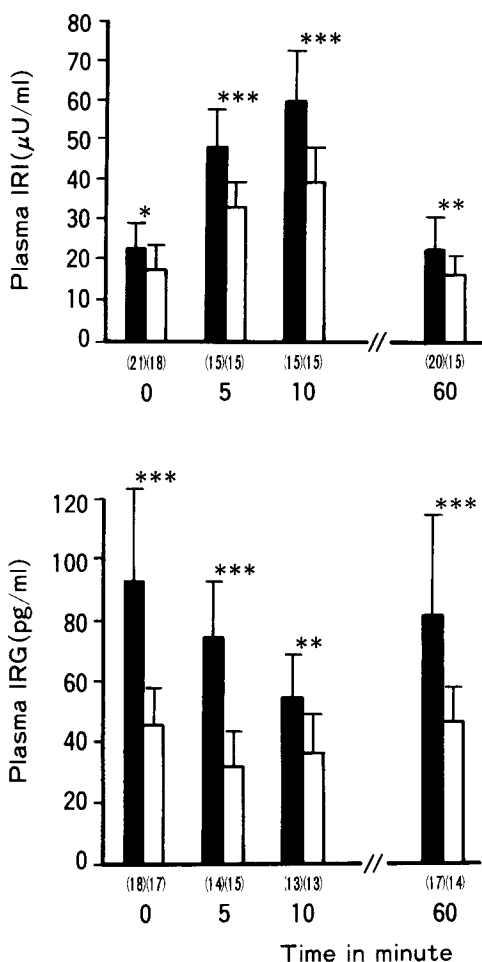

Fig. 2 Plasma IRI (top panel) and IRG (bottom panel) levels after intravenous glucose (1 g/kg) administration. ■, bile duct ligation; □, sham operation. Values are the mean ± SD, with the number of observation in parentheses. *, P < 0.05; **, P < 0.01; and ***, P < 0.001 for the differences from the control.

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Table 3   Contents of liver glycogen after intravenous glucose administration

Animals	Liver glycogen content			
	Fasted		60 min after glucose load	
	μmoles/g liver	μmoles/liver/100gBW	μmoles/g liver	μmoles/liver/100gBW
Control	1.6±1.1	5.0±3.6(10)	22.7±12.1 ⎤ *	73.4±42.4(18) ⎤ *
			⎟ *	⎟ *
BDL	1.1±0.5	5.6±2.6(8)	4.8±3.9 ⎦ *	24.4±19.4(14) ⎦ *

Values are the mean ± SD, with the number of ebservation in parentheses. Glycogen values are given in glucose equivalents. BW, body weight. Other symbols, see the legend to Table 1.

Table 4   Activities of key carbohydrate-metabolizing liver enzymes

Animals	Number of animal	Key enzyme activity (Unit/g liver)				GK+HK/G6Pase ratio
		GK	HK	GK + HK	G6Pase	
Control	15	1.48±0.21 ⎤ *	0.44±0.07 ⎤ *	1.92±0.22 ⎤	18.2±3.0 ⎤ *	0.11±0.22 ⎤ *
		⎟ *	⎟ *	⎟ *	⎟ *	⎟ *
BDL	20	0.44±0.22 ⎦ *	1.23±0.23 ⎦ *	1.66±0.32 ⎦	7.9±1.6 ⎦ *	0.21±0.08 ⎦ *

Values are the mean ± SD. *, P<0.05; * * *, P<0.001. GK, glucokinase; HK, hexokinase; G6Pase, glucose-6-phosphatase; BDL, see Table 1.

Table 5   Correlation coefficients between the glycogen content in liver and key enzyme activities and the K value 60 min after intravenous glucose administration

Animals	Number of animals	Key enzyme activities (Unit/g liver)				GK + HK/ G6Pase ratio	K value
		GK	HK	GK + HK	G6Pase		
Control	14	0.491	0.337	0.531	−0.283	0.591*	−0.731**
BDL	18	−0.441	−0.221	−0.442	0.130	−0.398	−0.400

*, P < 0.05; * *, P < 0.01.   Abbreviations: See Tables 1 and 4.

icteric livers were significantly lower than in the control livers. However, when the results were expressed on a whole liver basis, significant differences in glucose uptake or insulin extraction were not observed between the two groups. The glycogen deposition in the icteric liver remained still low, 52 % of the control value even it was calculated in terms of the whole liver. A positive correlation (r = 0.678) between the glycogen content and glucose uptake was observed (Fig. 3), although the rate of conversion from glucose to glycogen decreased markedly (48 % of the control) in the icteric livers (Table 6). Among key

carbohydrate-metabolizing enzymes determined in the perfused liver, GK and G6Pase activities decreased, while HK activity increased, as was observed in livers of rats with bile duct ligation. A positive correlations (r = 0.872) between glucose uptake and GK activity were observed in both groups (Fig. 4). No correlation between the glucose uptake and GK + HK or G6Pase activity was observed in either of the groups.

The lactate uptake and glucose production by livers perfused with 8.4 mM lactate added as a substrate are shown in Table 7. Lactate uptake and glucose production per unit liver weight in the

icteric livers were significantly lower than in the control livers (57 % and 44 % of the control, respectively). When the results were expressed in terms of the whole liver, no difference in the lactate uptake was observed between the two groups, although glucose production (72 % of the control) was significantly lower in the icteric group. Highly significant correlations between lactate uptake and glucose production existed in both groups as shown in Fig. 5, although the rate of the conversion of lactate to glucose (glucose production/1/2×lactate uptake) was significantly lower (77 % of the control) in the icteric livers, indicating an impaired gluconeogenesis.

Levels of glycolytic and gluconeogenic inter-

mediates in the perfused liver and in the liver after fasting without perfusion are listed in Table 8. The results of cross-over point analysis of these data by taking the values in the control group as 100 % are shown in Fig. 6. Slight increases in concentrations of G6P, F6P, pyruvate, lactate and the total of measured intermediates were observed in livers of bile duct-ligated rats before perfusion, although the changes were not statistically significant. The levels of total adenine nucleotides and ADP decreased significantly, and that of ATP to a small extent, in the icteric livers before perfusion. However, no changes were observed in the energy charge level.

In the livers perfused with glucose and insulin

Table 6   Glucose uptake, glycogen content and insulin extraction in the liver perfused with glucose and insulin

Animals	Number of animals	Glucose uptake		Glycogen content		Insulin extraction		Glycogen content/ Glucose uptake ratio
		μmoles/ g liver/h	μmoles 100gBW/h	μmoles/ g liver/h	μmoles/ 100gBW/h	%/ g liver/h	%/liver 100gBW/h	
Control	7	25.3±4.5	76.3±13.7	12.3±4.0	37.2±11.7	12.2±1.5	36.9±4.1	0.50±0.17
BDL	10	15.4±4.8	82.5±23.2	3.6±1.9	19.4±10.4	7.5±1.2	40.1±3.9	0.24±0.13

Values are the mean ± SD. Insulin extraction is expressed as a percentage obtained by dividing the difference between the pre- and post-perfusion values of insulin remained in the perfusate by the pre-perfusion value. **, $P < 0.01$; ***, $P < 0.001$.
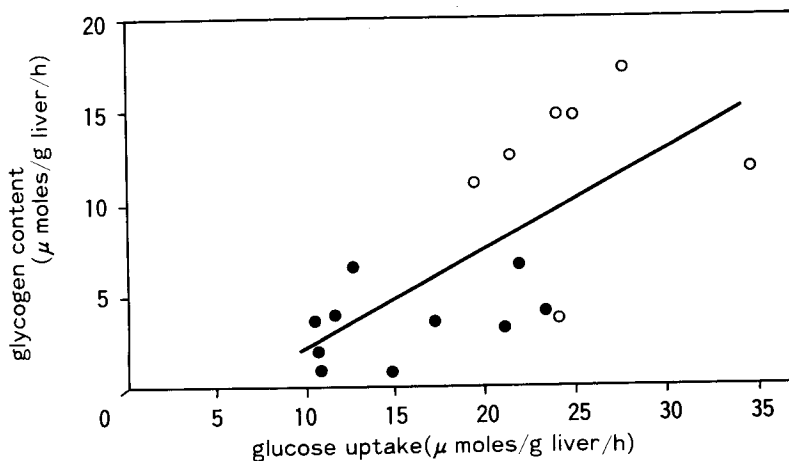

Fig. 3   Relationship between the glucose uptake and glycogen content in livers perfused with glucose and insulin. ●, livers from rats with bile duct ligation (N = 10); ○, control livers (N = 7). Correlation coefficient, r = 0.678; $P < 0.01$; regression equation, Y = 0.52X−3.00.

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as a substrate, increases in pyruvate and lactate levels, which were associated with an increase in FBP and a decrease in PEP, were observed in the icteric group, indicating enhanced glycolysis. In spite of the decrease in glucose uptake per unit liver weight in the icteric livers, G6P, F6P and G1P levels increased markedly, and the total concentration of intermediates also showed a significant increase to 1.5 times the level of the control. The ATP level in the icteric livers decreased significantly in spite of enhanced glycolysis, and the content of total adenine nu-

cleotides also decreased significantly. No significant difference in energy charge was observed between the two groups. In both groups, marked increases in the glycolytic intermediates and decreases in adenine nucleotides were seen in perfused livers as compared with preperfused livers.

In the livers perfused with lactate as a gluconeogenic substrate, the level of lactate and G6P increased, and the level of PEP and glucose decreased as a result of the bile duct ligation. The cross-over points observed between pyruvate and
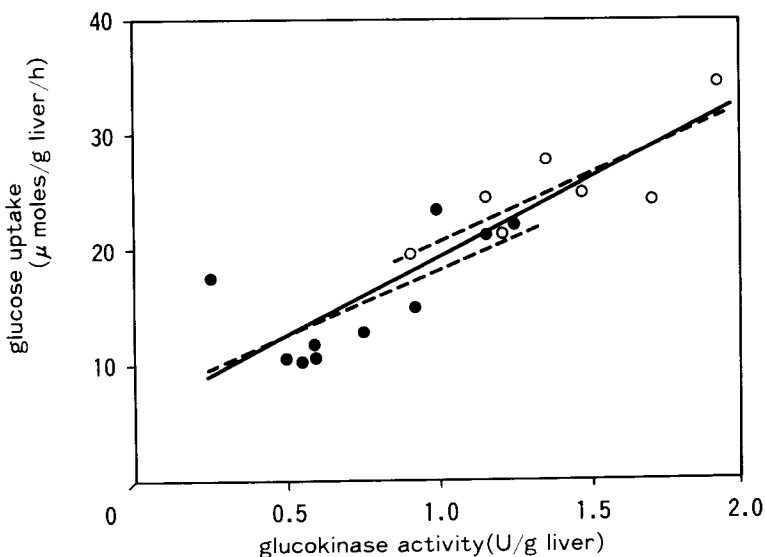

Fig. 4   Relationship between the glucokinase activity and glucose uptake in livers perfused with glucose and insulin. ●, livers from rats with bile duct ligation (N = 10); ○, control livers (N = 7). Correlation coefficient in the control group, r = 0.814; P < 0.05; regression equation, Y = 11.5X + 9.3. Correlation coefficient in the bile duct ligation group, r = 0.687; p < 0.05; regression equation, Y = 11.0X + 7.1. Correlation coefficient in both groups, r = 0.872; P < 0.001; regression equation, Y = 13.3X + 5.9.

Table 7   Lactate uptake and glucose production in the liver perfused with lactate

Animals	Number of animals	Lactate uptake		Glucose production		Glucose production/ Lactate uptake × 1/2 ratio
		μmoles/ g liver/h	μmoles/ liver/100gBW/h	μmoles/ g liver/h	μmoles/ liver/100gBW/h	
Control	10	46.5±5.3 ⎤ ** **	147.9±16.2	30.1±3.4 ⎤ ** **	95.3±6.5 ⎤ ** **	1.30±0.10 ⎤ ** **
BDL	11	26.5±6.9 ⎦	139.3±26.8	13.1±3.4 ⎦	68.5±12.0 ⎦	1.00±0.14 ⎦

Values are the mean ± SD.   Symbols, see the legend to Table 1.
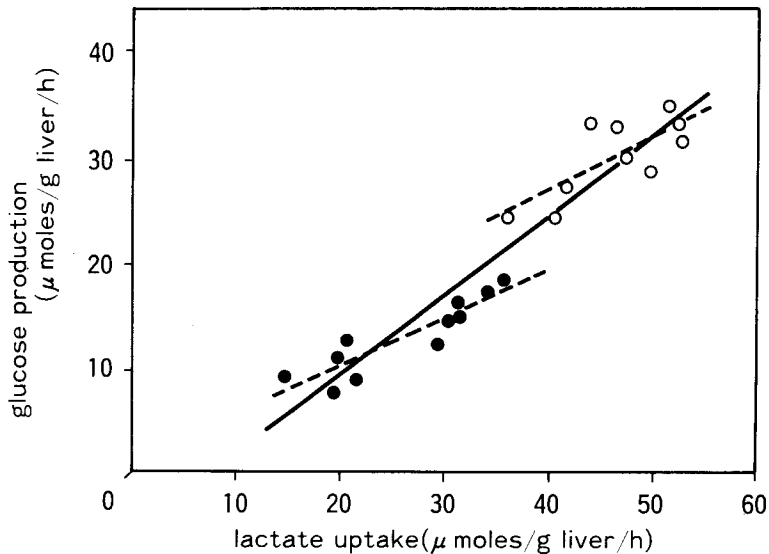
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Fig. 5   Relationship between the lactate uptake and glucose production in livers perfused with lactate. ●, livers from rats with bile duct ligation (N = 11); and ○, control livers (N = 10). Correlation coefficient in the control group, r = 0.756; P < 0.05; regression equation, Y = 0.49X + 0.74. Correlation coefficient in the bile duct ligation group, r = 0.909; P < 0.001; regression equation, Y = 0.45X + 1.11 Correlation coefficient in both groups, r = 0.954; P < 0.001; regression equation, Y = 0.74X − 5.61.

Table 8   Levels of intermediary metabolites in livers before and after perfusion with glucose and insulin or lactate

Metabolites (μmoles/g liver)	Pre-perfusion		Perfusion with glucose and insulin		Perfusion with lactate	
	Control (5)	BDL (5)	Control (7)	BDL (10)	Control (10)	BDL (11)
G1P	0.025±0.006	0.023±0.007	0.014±0.002	0.022±0.005 ^{b}	0.020±0.007	0.021±0.008
G6P	0.091±0.020	0.116±0.019	0.085±0.018	0.155±0.033 ^{c}	0.044±0.015	0.074±0.017 ^{c}
F6P	0.023±0.005	0.024±0.006	0.028±0.007	0.040±0.008 ^{b}	0.020±0.007	0.017±0.007
FBP	N.D.	N.D.	0.017±0.006	0.018±0.005	N.D.	N.D.
GAP/DHAP	N.D.	N.D.	0.046±0.011	0.050±0.014	0.019±0.010	0.016±0.009
PEP	0.062±0.034	0.059±0.035	0.097±0.027	0.073±0.030	0.062±0.023	0.043±0.027
Pyruvate	0.021±0.008	0.025±0.010	0.176±0.047	0.201±0.037	0.083±0.048	0.112±0.079
Lactate	0.970±0.345	1.150±0.159	1.710±0.546	2.682±0.586 ^{b}	0.852±0.398	1.610±0.711 ^{a}
Total	1.193±0.337	1.398±0.160	2.172±0.623 †	3.240±0.629 ^{b}†	1.099±0.450	1.894±0.751 ^{a}
ATP	2.046±0.274	1.859±0.144	2.015±0.295	1.651±0.266 ^{a}	1.475±0.363 *	1.276±0.238 †
ADP	0.884±0.134	0.608±0.081 ^{b}	0.645±0.147	0.703±0.092	0.515±0.197	0.498±0.199
AMP	0.567±0.156	0.508±0.215	0.354±0.127	0.227±0.102 ^{a}	0.852±0.341	0.613±0.237
Total	3.497±0.218	2.974±0.382 ^{a}	3.014±0.328	2.585±0.286 ^{a}	2.842±0.410	2.386±0.221 ^{b}
Energy charge	0.711±0.042	0.732±0.043	0.775±0.035 *	0.775±0.045	0.612±0.124	0.640±0.094

Values are the mean ±S.D, with the number of observations in parentheses. N.D., not detectable. *a*, P < 0.05; *b*, P < 0.01; *c*, P < 0.001, as compared with sham-operated controls. †, P < 0.001, as compared with pre-perfusion BDL levels. *, P < 0.05, as compared with pre-perfusion control levels.

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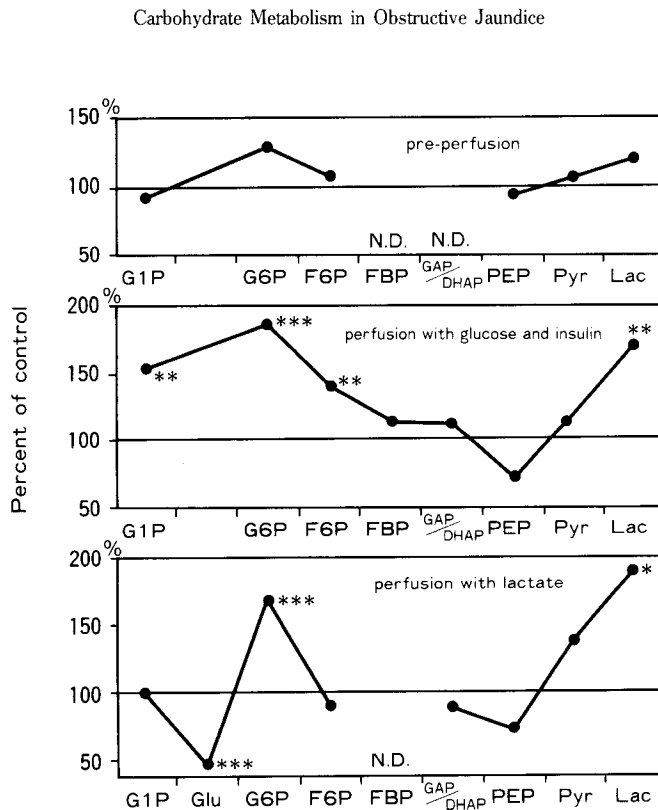
Fig. 6   Effect of bile duct ligation on levels of intermediary metabolites in livers before and after perfusion with glucose and insulin, or lactate alone. Data are expressed as a percentage of control values obtained with sham-operated rats. Glu, glucose ; Pyr, pyruvate; Lac, lactate; N.D., not detectable.   *, P < 0.05; **,P < 0.01; and ***, P < 0.01, as compared with sham-operated controls.

PEP and between G6P and glucose (Fig. 6) indicated a metabolic block at the levels of phosphoenol pyruvate carboxykinase (PEPCK) and G6Pase reactions. The concentration of total intermediates increased markedly in the ligated group. No significant difference in the ATP level was observed following the lactate perfusion between the icteric and the control groups, although ATP levels in both groups decreased significantly as compared with the pre-perfusion levels. The level of total adenine nucleotides decreased in the icteric group, although no significant difference in energy charge was observed between the two groups.

In summarizing the above results, the liver of rats with obstructive jaundice had decreased glucose uptake per unit liver weight in association with lowered GK activity. However, no difference in the glucose uptake and insulin extrac-

tion from the control group was observed when the results were expressed on a whole liver basis. Evaluation of the changes in metabolite levels revealed increased glycolysis, and decreased gluconeogenesis and glycogenesis. The latter two indicate a loss of differentiated functions of the liver.

Discussion

The most significant result of the present study was that no decrease in glucose tolerance was observed, unlike the impaired glucose tolerance in human obstructive jaundice (5, 6). In fact, the rate of fractional glucose removal in the IVGTT was increased, and the glucose tolerance was improved. Similar results were obtained by Record et al. (7) in an IVGTT on jaundiced rats

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prepared by bile duct ligation. The rate of fractional glucose removal in the IVGTT is known to correlate well with the acute insulin response (29, 30), since only 20 % of the intravenously loaded glucose is taken up by the splanchnic bed (31). The rest is taken up by peripheral tissues as a result of the action of insulin. Therefore, the increase in the rate of fractional glucose removal observed in the icteric group may be explained by the observed hyperinsulinemia. The difference in the insulin level between the pre-loading and early phase (5 and 10 min) of the IVGTT was higher in the icteric group than in the control group, suggesting enhanced insulin secretion in the former group. In fact, the insulin extraction by the whole liver was not different between the icteric group and the control group, as demonstrated by the perfusion of liver with glucose and insulin, indicating that the hyperinsulinemia observed in the icteric group is due to enhanced insulin secretion by islet cells rather than reduced insulin degradation by the liver. On the basis of electron microscopic observations of pancreatic islet cells and measurement of fasting plasma IRI levels in rats with bile duct ligation, Shim *et al.* (32) confirmed the hyperfunction of islet B cells and hyper-basal secretion of insulin two weeks after bile duct ligation, thus supporting the present results.

The suppression of NEFA following glucose load, which depends on insulin secretion, was more marked in the icteric group than in the control group. The high glucagon level observed in the icteric group also responded well to the glucose administration, resulting in significant decreases. These results suggest that the action of insulin was predominant over that of glucagon in the bile duct-ligated rats even when the glucagon level was elevated. Record *et al.* (7) observed a reduction of fasting ketone bodies in rats upon bile duct ligation, a phenomenon which can also be explained by the insulin-dependent suppression of NEFA release. Elevated plasma levels of insulin and glucagon have been reported not only in obstructive jaundice (8, 32) but also in

hepatitis (33, 34) and liver cirrhosis (35-38). Accordingly, increased levels of these hormones are considered to be a change common to various types of hepatic injuries, although the mechanisms involved have not been elucidated.

In parenchymal liver injuries caused by experimental hepatotoxins, acute and chronic hepatitis and liver cirrhosis, the activities of prototype or fetal-type liver enzymes, such as HK, G6PD, PFK and PK-M_2, increase and those of adult-type liver enzymes, such as GK, G6Pase and FBPase, decrease in the injured livers (2, 3). Similar enzyme alterations have been demonstrated by Kurose (1) in livers of rats with obstructive jaundice induced by bile duct ligation. On the basis of these facts, Kurose has proposed that in obstructive jaundice the liver is affected not only by cholestasis but also by parenchymal liver injury. Taketa *et al.* (4) described the importance of decreased activity of liver GK in lowering the glucose tolerance in hepatic injury. However, in the present study the glucose tolerance in the IVGTT in rats with obstructive jaundice was enhanced. The increased glucose tolerance may be accounted for mainly by increased glucose uptake by peripheral tissues due to hypersecretion of insulin, and thus the reduced GK activity was considered not to reflect a reduction in glucose tolerance. The results of the present liver perfusion study with glucose at a concentration as high as 22 mM revealed that hepatic glucose uptake had a high correlation only with GK activity in both the icteric group and the control group, confirming the importance of GK activity to the uptake of glucose by the liver (4, 16). In view of the fact that the Km of GK is 10 mM in rats (39), the glucose concentration in the portal vein during the IVGTT was not sufficiently high to exert a GK effect on hepatic phosphorylation of glucose.

Glycogen synthesis is one of the specific functions of the liver, and the glycogen content is reduced in liver tissues affected by hepatitis, liver cirrhosis and other pathological conditions (40, 41). In rats with bile duct ligation, glycogen

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content decreased to 21% and 29% of the control values expressed on the basis of unit liver weight when determined after the IVGTT and liver perfusion with added glucose, respectively. Considering that a correlation was observed between glucose uptake and glycogen content in perfused livers, the reduced glycogen content of the liver observed in the icteric group is probably related to decreased glucose uptake due to lower GK activity. On the other hand, the rate of conversion of glucose to glycogen in the icteric group was also decreased to 48% of the control as revealed by a decreased ratio of glycogen content to glucose uptake, indicating that glycogenesis was impaired. In other words, the lowered glycogen content observed in the icteric group may be ascribable not only to the decrease in hepatic glucose uptake but also to the decreased formation of glycogen from glucose.

Liver glycogen content in the control group in the IVGTT experiment showed a negative correlation with the K value and a positive correlation with the phosphorylating activity indicated by the ratio of (GK + HK)/G6Pase. HK has a low Km (39); thus it should have been saturated before glucose loading. The glucose uptake (phosphorylation) by the liver in response to the change in blood sugar level after the IVGTT might depend less upon glucose concentration when GK activity is reduced. Conversely, the glycogen content in the control liver with high GK activity might have been increased as a result of the increased hepatic uptake of glucose by the GK reaction with higher concentrations of glucose in the hepatic portal vein as a result of the low K value; namely, utilization of glucose by peripheral tissues would be reduced. The glycogen content in the ligated liver in the IVGTT showed no correlation with either the K value or (GK + HK)/G6Pase ratio, probably due to the impaired conversion of phosphorylated glucose to glycogen as mentioned below.

Reduced glycogen content of the liver can be brought about either by a reduction in glycogen synthesis or by an increase in glycogenolysis.

However, no satisfactory investigation has been carried out on the mechanism of lowering the liver glycogen content by various hepatic injuries. Kobayashi (42) demonstrated a marked increase in the active form of liver phosphorylase in rats with acute hepatic injuries caused by CCl_4 and galactosamine administration, suggesting potential hyperglycogenolysis in injured livers. Glycogen synthesis in injured livers has not been determined, although several lines of evidence suggest that the glycogenesis of injured livers is reduced, as may be seen in CCl_4 hepatic injury (43) and viral hepatitis (44). In the present study, the concentrations of G6P and G1P in the glucose-perfused livers increased markedly, suggesting enhanced glycogen degradation in addition to the reduced glucose phosphorylation due to low GK activity. Van Noorden et al. (45) confirmed the existence of increased glycogen phosphorylase activity in rat liver parenchyma after bile duct ligation, thus supporting the present contention.

In the glucose-supplemented perfusion experiment, elevated pyruvated and lactated levels were observed in association with decrease PEP and increase FBP, obviously indicating enhanced glycolysis in rats with bile duct ligation. The existence of a cross-over point between PEP and pyruvate and of a steep gradient between F6P and FBP indicates increased metabolic rates at PK and PFK steps in the cholestatic liver. In fact, Kurose (1) reported elevated activities of PK and PFK in the liver of rats with obstructive jaundice. On the other hand, impairment of gluconeogenesis in cholestatic liver was indicated by the lactate-supplemented perfusion experiment, in which both lactate uptake and glucose production were markedly decreased with a reduced rate of conversion from lactate to glucose. The existence of cross-over points between pyruvate and PEP as well as between G6P and glucose may imply that PEPCK and G6Pase activities are reduced in the icteric liver. Changes in the activities of PEPCK, pyruvate carboxylase and other gluconeogenic enzymes have not been examined in the liver of animals with obstructive

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jaundice. However, there has been a report (46) of reduced activities of PEPCK and pyruvate carboxylase in addition to G6Pase and FBPase in injured liver.

Obstructive jaundice causes mitochondrial damage resulting in the derangement of energy metabolism (6, 47, 48). Increased levels of bilirubin appear to be responsible for the uncoupling of oxidative phosphorylation (49, 50). The present results obtained in liver perfusion with glucose and insulin revealed a significant decrease in the hepatic ATP level in cholestatic rats, suggesting the presence of mitochondrial damage. The content of total adenine nucleotides decreased simultaneously, but the energy charge remained unchanged. It has been shown that a lowered ATP or increased AMP level activates PK and PFK and inhibits FBPase activity, resulting in activation of the glycolytic system. The increased glycolytic activity in the livers of rats with bile duct ligation is evident from the results of metabolite analysis and may contribute to the consumption of glycogen. Increased glycolysis may partially compensate for the lowered ATP production due to mitochondrial dysfunction and maintain the energy charge at a normal level. The increased levels of total glycolytic intermediates reflect the consumption of inorganic phosphate through phosphorylation of glucose, and this in turn leads to the lowering of total adenine nucleotides via AMP degradation by activation of adenosine deaminase activity (51). Thus, the decreased adenine nucleotide level may also be accounted for by the accumulation of glycolytic intermediates.

In summary, uptake of glucose and its conversion to glycogen were reduced in the cholestatic liver in close association with altered activities of some related enzymes, although the total amount of glucose utilized in the whole rat was not reduced due to increased utilization by the peripheral tissues.

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