Insulin Requirements for Hepatic Regeneration Following Hepatectomy

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On the basis of changes in the adenine nucleotide and mitochondrial metabolism of the remnant liver, insulin requirements for hepatic regeneration were studied in diabetic rats treated with varying amounts of alloxan. Mildly diabetic rats with less than 30% inhibition in maximal portal insulin response to oral glucose load, showed a parabolic glucose tolerance pattern and could tolerate partial hepatectomy. Whereas, severely diabetic rats with more than 45% inhibition showed a linear glucose tolerance pattern and died within 24 hours after partial hepatectomy. In the former rats, the energy charge (ATP + ½ADP/ATP + ADP + AMP) levels of the remnant liver decreased slightly at an early period after partial hepatectomy but could be restored rapidly to normal levels with a concomitant rise of oxidative phosphorylation in remnant liver mitochondria. In contrast, the energy charge levels in the latter groups fell more markedly and could not be restored, because of insufficient enhancement of mitochondrial oxidative phosphorylation. It is suggested that an enhancement in mitochondrial phosphorylative activity of the remnant liver following partial hepatectomy is inhibited in proportion to the severity of impaired insulin secretion, resulting in a decrease of the potential functional capacity of liver.

AN ENHANCEMENT of mitochondrial phosphorylative activity occurs with a concomitant decrease in the energy charge (ATP + ½ ADP/ATP + ADP + AMP) of the remnant liver[17] and is requisite for later increase in nuclear DNA synthesis in regenerating liver.[23] Such an enhancement is induced when an elevated level of portal factor is available to respiratory assemblies.[14] Evidence has accumulated indicating that insulin plays an important role as a portal factor in the mechanism by which the portal blood controls oxidative phosphorylation of the liver mitochondria.[15,16] Also, changes in mitochondrial phosphorylative activity during regeneration are associated with characteristic changes in glucose intolerance pattern.[7] Hepatectomy in patients with severe glucose intolerance or impaired insulin secretion results in high mortality.[21] However, quantitative information is not available concerning insulin requirement of regenerating liver. These results led us to show how the mitochondrial oxidative phosphorylation and the energy charge of liver change after partial hepatectomy in diabetic rats with varying degrees of impairement of insulin secretion.

In this study, evidence will be presented that the decrease of energy charge in the remnant liver following partial hepatectomy is augmented with insufficient enhancement of mitochondrial phosphorylative activity due to impaired insulin secretion, and that the more severe the impaired insulin secretion, the lower the potential regenerative capacity of liver.

Materials and Methods

Treatment of Experimental Animals

Adult male Wistar strain rats weighing 200 gm were maintained on Clea CE-2 (Nippon Haigoshiryo Co. Ltd.) and water ad libitum 2 weeks before treatment. Five groups of diabetic rats (group A, B, C, D and E) were induced by intravenous injection of 2.8% solution of alloxan monohydrate (Eastman Organic Chemicals)

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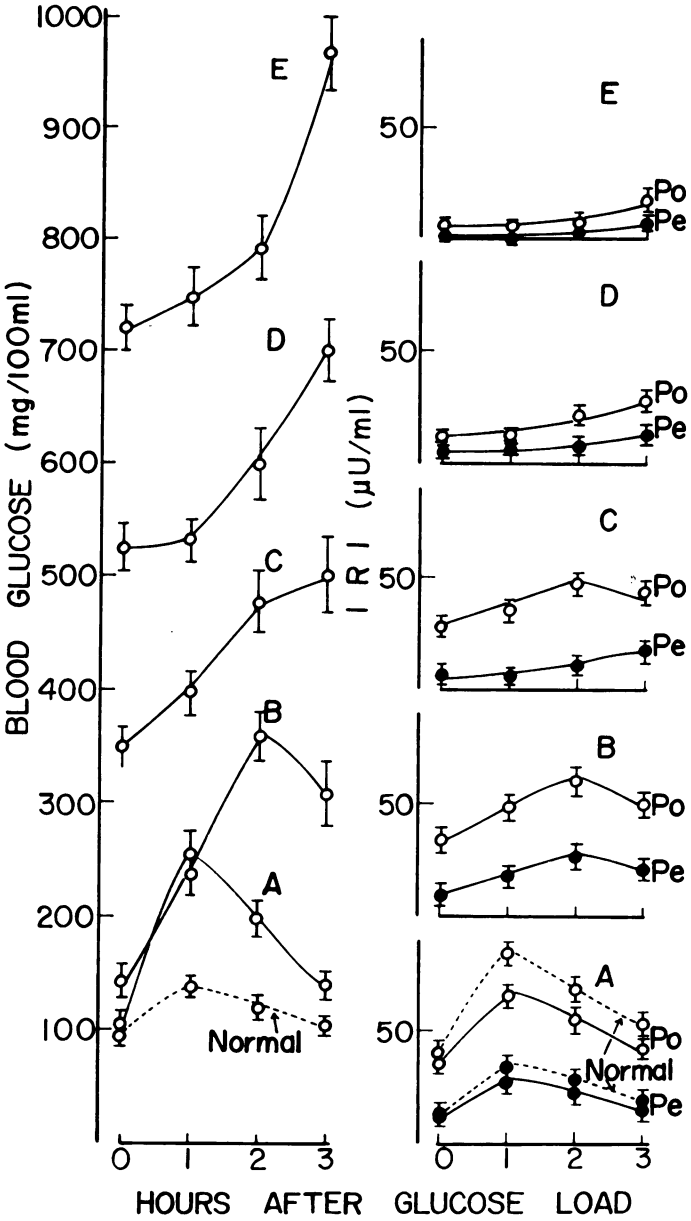

FIG. 1. Glucose tolerance (left) and Insulin Response (right) to an oral glucose load in 5 groups of diabetic rats treated with varying amounts of alloxan. Vertical bars indicate standard error of the mean of 8 to 10 animals. **A**, group A of diabetic rats treated with 35 mg/kg of alloxan; **B**, group B of diabetic rats treated with 49 mg/kg of alloxan; **C**, group C of diabetic rats treated with 63 mg/kg of alloxan; **D**, group D of diabetic rats with non-fatty livers treated with 70 mg/kg of alloxan; **E**, group E of diabetic rats with fatty livers treated with 70 mg/kg of alloxan. Po, portal vein insulin. Pe, peripheral vein insulin.

at a dose of 35, 49, 63 and 70 mg/kg of body weight. The animals were fasted for 15 hours before alloxan injection. Rats were considered diabetic if they exhibited polydipsia, polyuria with positive urine sugar (TES TAPE; Lilly & Co. Ind.), maximal blood sugar over 250 mg/100 ml after oral glucose load and blood hydroxybutylate (determined by enzymatic method[13]) over 2.10 mM. Urine ketone was determined by Ketostix (Ames Division, Miles-Sankyo Co. Ltd.). Hepatectomy and sham operation were performed 48 hours after alloxan injection at about 10 a.m.

Partial hepatectomy was performed under ether anesthesia in the conventional manner as originally described by Higgins and Anderson.[5] Fifteen hours before and after operation rats were fasted. In sham operated control rats laparotomy and separation of the median and left lateral lobes of the liver from those surrounding ligaments were carried out.

Glucose Tolerance Test

In the glucose tolerance test (GTT) a 50% solution of glucose (3 g/kg of body weight) was administered intragastrically after a fasting period of 15 hours. Blood samples were drawn at 0, 1, 2 and 3 hours. Blood glucose was determined by the *o*-toluidine method.[6] Plasma immunoreactive insulin (IRI) was estimated according to radioimmunoassay methods.[4]

Assays of Adenine Nucleotides

The liver was clamped and frozen in situ with stainless steel tongs precooled with liquid nitrogen. The frozen tissue was removed and immersed in liquid nitrogen through which CO had been bubbled. The procedure was completed within 10 seconds. The frozen tissue was powdered with a liquid nitrogen cooled stainless steel mortar and pestle in the liquid nitrogen bath. The powdered tissue was weighed and homogenized in 3 volumes of cold 5% perchloric acid at 0°. The supernatant was adjusted to pH 6.0 with cold 60% K_2CO_3 and recentrifuged at 10,000 g for 15 minutes at 0°. The amounts of ATP, ADP and AMP were enzymatically measured.[2]

Assays of Oxidative Phosphorylation of Liver Mitochondria

The preparation of liver mitochondria and the assays of oxygen consumption, phosphorylative activity and the contents of respiratory carriers were done by methods described elsewhere.[18] The respiratory control ratio was calculated from the polarographic tracing by the method of Chance[3] from the equation, RCR = state 3 respiration rate (in the presence of ADP)/state 4 respiration rate (after exhaustion of ADP). Since it has been found that, in alloxan diabetic rats cytochrome, concentrations decrease when expressed per mitochondrial protein,[10,12] the oxidative and phosphorylative activities are expressed relative to cytochrome a ($+a_3$) concentrations. The value is expressed as the moles of oxygen used (or ATP

TABLE 1. *Effect of Increasing Amounts of Injected Alloxan on Rats*

Group	Fasting blood sugar (mg/100 ml)	Blood hydroxy-butylate (mM)	Urine sugar	Urine ketone	Fatty liver	Survival Rate: 48 hours after alloxan injection	Survival Rate: 12 days after alloxan injection
Normal rats (20)*	101 ± 3†	1.25 ± 0.22	(−)	(−)	(−)	–	–
Group A (20) (35 mg/kg alloxan)	103 ± 2	2.10 ± 0.04	⅛%	(−)	(−)	100%	90%
Group B (20) (49 mg/kg alloxan)	142 ± 9	2.10 ± 0.72	⅛%	(−)	(−)	100%	90%
Group C (20) (63 mg/kg alloxan)	360 ± 13	2.37 ± 0.21	½%	(++)	(−)	95%	55%
Group D (20) (70 mg/kg alloxan)	520 ± 18	4.96 ± 0.75	½%	(+++)	(−)	70%	10%
Group E (20) (70 mg/kg alloxan)	712 ± 25	5.55 ± 0.83	2 %	(+++)	(++)		

* Figures in parenthesis indicate numbers of animals.
† Means ± SEM.

formed) per second divided by the moles of cytochrome $a(+a_3)$. Protein was determined by the method of Lowry et al.[11] with crystalline bovine serum albumin as standard.

Results

Figure 1 shows glucose tolerance and insulin response to oral glucose load in 5 groups of diabetic rats treated with varying amounts of alloxan. In the diabetic rats of group A treated with 35 mg/kg of alloxan, the fasting blood glucose levels were within normal limits but the maximal blood glucose level reached approximately 260 mg/100 ml after an oral glucose load. The portal and peripheral vein insulin responses at the maximum to glucose load were suppressed to about 78% and 79% of normal controls, respectively. In the diabetic rats of group B treated with 49 mg/kg of alloxan, the fasting blood glucose levels increased slightly and the maximal peak of blood glucose level was observed at 2 hours. The portal and peripheral vein insulin responses at the maximum to glucose load were suppressed to 72% and 75% of controls, respectively. Fasting portal and peripheral vein insulin levels in groups A and B were within normal limits. In these two groups of mildly diabetic rats, although there was no ketonuria, glucosuria developed and blood hydroxybutylate levels increased to a significantly high level (Table 1). Most of these animals lived longer than 12 days after alloxan injection. In diabetic rats (group C) treated with 63 mg/kg of alloxan, fasting blood glucose levels were about 360 mg/100 ml and blood hydroxybutylate level was 2.37 mM with positive ketonuria. The blood glucose level increased after an oral glucose load and reached about 500 mg/100 ml at 3 hours. The portal and peripheral vein insulin responses to glucose load were inhibited to 55% and 50% of controls, respectively. Fasting portal and peripheral vein insulin levels were 78% and 67% of controls. About 55% of these diabetic rats died within 12 days after alloxan injection. When 70 mg/kg of alloxan were given, the diabetic rats were divided into two groups according to the absence or the presence of fatty liver: the mean fasting blood glucose levels of the diabetic rats with non-fatty livers were about 520 mg/100 ml (group D) and those with fatty livers were about 712 mg/100 ml (group E). Fasting portal and peripheral vein insulin levels and insulin responses at the maximum to glucose load were inhibited severely. Glucosuria and ketonuria developed concomitantly with high blood hydroxytubylate (5.55 mM) in these groups. Only 10% of these diabetic rats treated with 70 mg/kg of alloxan lived beyond 12 days. Since the blood glucose curves of groups A and B showed a return of blood glucose level toward normal levels within 3 hours, they are classified as parabolic GTT patterns. The blood glucose levels of groups C, D and E increased linearly for more than 3 hours after an oral glucose load. This type of response is called a linear GTT pattern.

Figure 2 shows the survival rate of diabetic rats after partial hepatectomy. In groups A and B the survival rates of hepatectomized and sham operated groups were over 70% at 10 days after operation. In group C the survival rate fell rapidly to zero within 4 days after partial hepatectomy, and in the sham operated group the survival rate was 50% at 10 days. In groups D and E the survival times were short following either hepatectomy or sham operation, especially after partial hepatectomy.

Figure 3 shows changes in the energy charge of remnant liver after partial hepatectomy in diabetic rats. In normal rats, the energy charge levels of the remnant

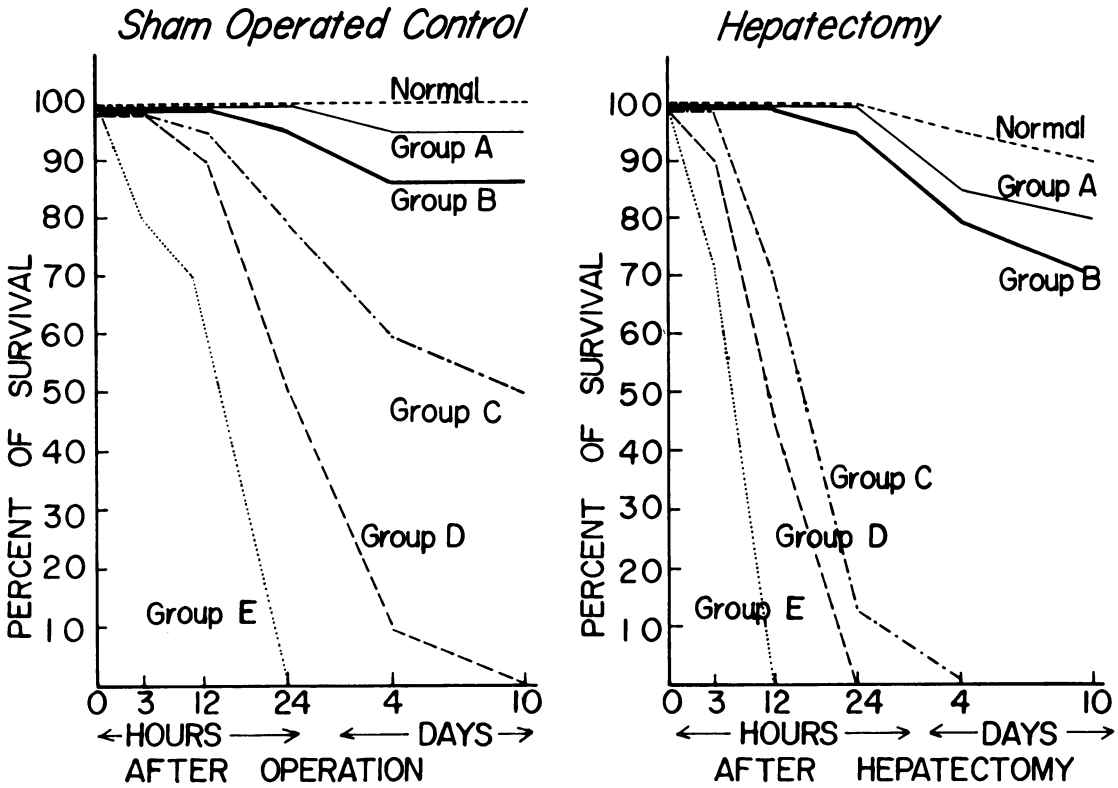

FIG. 2. The survival rate of 5 types of alloxan diabetic rats following partial hepatectomy.

liver decreased significantly ($P < 0.05$) from 0.846 ± 0.001 to 0.839 ± 0.002 at 3 hours after partial hepatectomy and returned rapidly to normal level within 12 hours. In groups A and B of diabetic rats, the energy charge of the remnant liver decreased significantly ($P < 0.05$) from 0.864 ± 0.003 to 0.817 ± 0.007 and returned to normal level within 12 hours after partial hepatectomy. On the other hand, in groups C, D and E

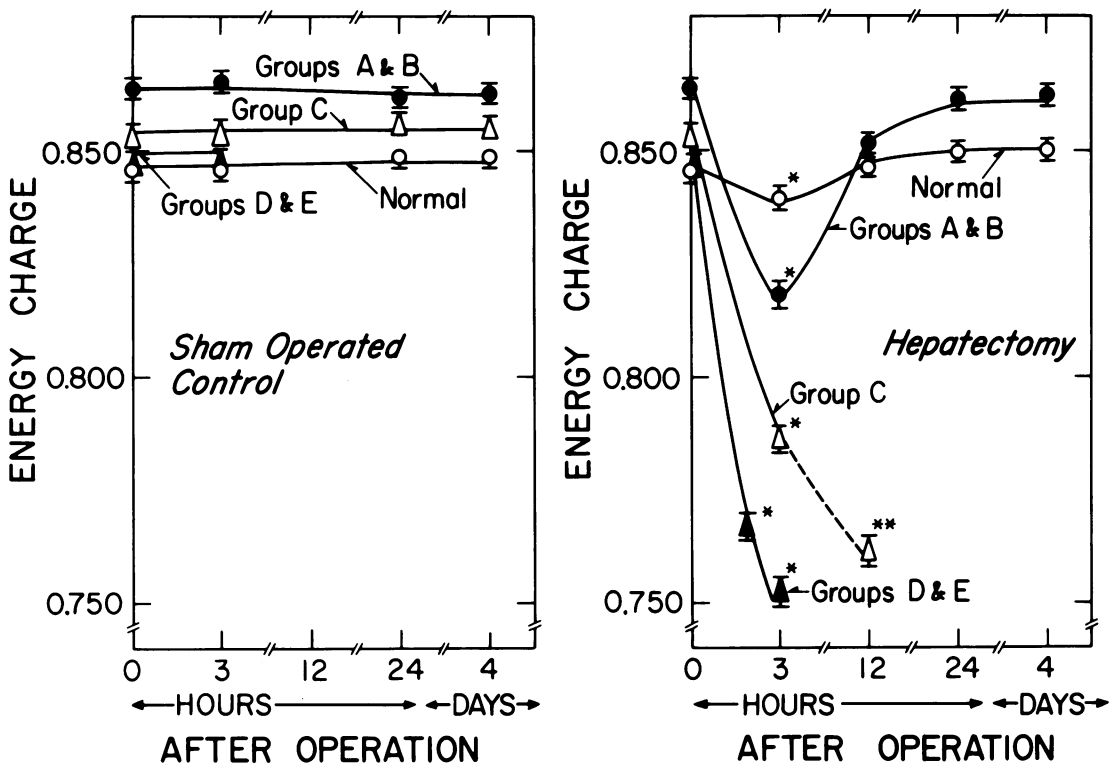

FIG. 3. Changes in the energy charge of the remnant liver in 5 types of diabetic rats following partial hepatectomy. *Significantly lower than the corresponding sham operated group. **Only 2 animals were studied.

of diabetic rats, the energy charge fell rapidly to remarkably lower level ($P < 0.01$) (0.785 ± 0.012 in group C and 0.752 ± 0.011 in group D and E) at 3 hours after partial hepatectomy, continued to fall and did not show any tendency to recover. In sham operated diabetic rats, however, there occurred no significant changes in the energy charge levels during the period tested.

Table 2 shows the details of the changes in the concentrations of adenine nucleotides of the remnant liver after partial hepatectomy. In groups A and B of diabetic rats, only slight reduction of ATP with a concomitant rise of ADP in the remnant liver was observed at an early stage after partial hepatectomy with rapid recovery to normal within 12 hours. The AMP and total nucleotide levels remained within normal concentrations. On the other hand, in groups C, D and E both ATP and total nucleotide concentrations decreased to significantly lower levels with a concomitant rise in both ADP and AMP concentrations.

Changes in phosphorylative activity per unit of cytochrome a ($+a_3$) of mitochondria from the remnant liver after partial hepatectomy in diabetic rats, and the details of the changes in mitochondrial oxidative phosphorylation are shown in Fig. 4 and Table 3. In groups A and B of diabetic rats oxidative and phosphorylative activities per unit of cytochrome a ($+a_3$) of liver mitochondria were not significantly different from normal controls. In group C phosphorylative activity per unit of cytochrome a ($+a_3$) decreased slightly. In group D oxidative and phosphorylative activity per unit of cytochrome a ($+a_3$) and respiratory control ratio were inhibited significantly, although the P/O ratio remained within normal limits. In group E all of these levels were inhibited severely. After partial hepatectomy oxidative and phosphorylative activities per unit of cytochrome a ($+a_3$) of the remnant liver in groups A and B enhanced to significantly higher levels than those in sham operated normal rats shortly after operation and then gradually declined. The oxidative and phosphorylative activities per unit of cytochrome a ($+a_3$) in the remnant livers of groups C, D and E did not, however, reach the levels of sham operated normal rats.

TABLE 2. *Changes of the Concentrations of Adenine Nucleotides of Remnant Liver Tissues in Five types of Alloxan Diabetic Rats following Partial Hepatectomy.*

Adenine Nucleotides	Groups	Before Operation	3 hr Postop		12 hr Postop		24 hr Postop		4 Days Postop	
			Sham	Hepatectomy	Sham	Hepatectomy	Sham	Hepatectomy	Sham	Hepatectomy
ATP (μmoles/ gm/wet wt)	Normal	2.66 ± 0.77 (8)	2.71 ± 0.09 (4)	2.38 ± 0.08 (6)	—	2.30 ± 0.12 (6)	2.82 ± 0.08 (4)	2.36 ± 0.09 (6)	2.80 ± 0.11 (4)	2.59 ± 0.15 (4)
	A & B	2.27 ± 0.11 (8)	2.41 ± 0.12 (4)	2.08 ± 0.14* (6)	—	2.47 ± 0.11 (6)	2.37 ± 0.12 (4)	2.68 ± 0.12 (6)	2.60 ± 0.12 (4)	2.66 ± 0.10 (4)
	C	2.53 ± 0.12 (8)	2.62 ± 0.13 (4)	1.95 ± 0.12* (6)	—	1.78 ± 0.01 (2)	2.62 ± 0.10 (4)	—	2.62 ± 0.10 (4)	—
	D & E	2.43 ± 0.08 (8)	2.44 ± 0.10 (4)	1.79 ± 0.08* (6)	—	—	—	—	—	—
ADP (μmoles/ gm/wet wt)	Normal	0.78 ± 0.03 (8)	0.75 ± 0.05 (4)	0.84 ± 0.03 (6)	—	0.73 ± 0.06 (6)	0.83 ± 0.05 (4)	0.73 ± 0.06 (6)	0.93 ± 0.50 (4)	0.74 ± 0.03 (4)
	A & B	0.59 ± 0.05 (8)	0.54 ± 0.05 (4)	0.85 ± 0.07* (6)	—	0.61 ± 0.07 (6)	0.53 ± 0.07 (4)	0.54 ± 0.03 (6)	0.55 ± 0.03 (4)	0.58 ± 0.04 (4)
	C	0.74 ± 0.06 (8)	0.68 ± 0.04 (4)	0.92 ± 0.05* (6)	—	0.91 ± 0.05 (2)	0.72 ± 0.05 (4)	—	0.69 ± 0.04 (4)	—
	D & E	0.71 ± 0.02 (8)	0.73 ± 0.04 (4)	0.97 ± 0.04* (6)	—	—	—	—	—	—
AMP (μmoles/ gm/wet wt)	Normal	0.16 ± 0.007 (8)	0.17 ± 0.012 (4)	0.11 ± 0.013 (6)	—	0.11 ± 0.014 (6)	0.16 ± 0.012 (4)	0.11 ± 0.013 (6)	0.15 ± 0.011 (4)	0.15 ± 0.013 (4)
	A & B	0.10 ± 0.012 (8)	0.14 ± 0.010 (4)	0.14 ± 0.008 (6)	—	0.17 ± 0.010 (6)	0.15 ± 0.009 (4)	0.19 ± 0.009 (6)	0.18 ± 0.012 (4)	0.19 ± 0.008 (4)
	C	0.13 ± 0.011 (8)	0.14 ± 0.011 (4)	0.19 ± 0.012* (6)	—	0.24 ± 0.012 (2)	0.13 ± 0.011 (4)	—	0.15 ± 0.009 (4)	—
	D & E	0.15 ± 0.010 (8)	0.14 ± 0.011 (4)	0.19 ± 0.012* (6)	—	—	—	—	—	—
Total Nucleotide (μmoles/ gm/wet wt)	Normal	3.60 ± 0.12 (8)	3.63 ± 0.13 (4)	3.32 ± 0.14 (6)	—	3.09 ± 0.11 (6)	3.80 ± 0.12 (4)	3.20 ± 0.08 (6)	3.79 ± 0.12 (4)	3.48 ± 0.10 (4)
	A & B	2.96 ± 0.15 (8)	3.09 ± 0.14 (4)	3.07 ± 0.15 (6)	—	3.26 ± 0.09 (6)	3.05 ± 0.09 (4)	3.41 ± 0.13 (6)	3.33 ± 0.15 (4)	3.46 ± 0.12 (4)
	C	3.40 ± 0.16 (8)	3.44 ± 0.53 (4)	3.07 ± 0.15* (6)	—	2.93 ± 0.12 (2)	3.47 ± 0.11 (4)	—	3.47 ± 0.08 (4)	—
	D & E	3.29 ± 0.08 (8)	3.31 ± 0.09 (4)	3.02 ± 0.69* (6)	—	—	—	—	—	—

The results shown are mean values ± SEM.
Values in parenthesis indicate numbers of animals.
* Significant statistically compared to corresponding sham operated animals. ($P < 0.05$).

Discussion

A continuous supply of ATP, mainly from oxidative phosphorylation, is essential to maintain the diverse functions of the hepatocytes. Normally, the rate of formation of ATP in the liver cells is equal to the rate of use of ATP, and intracellular ATP is maintained at a constant level. In this dynamic steady state, the concept of the energy charge proposed by Atkinson[1] as an indicator of cellular energy status is useful for understanding the cell's energy flow between energy-utilizing reactions and energy-generating reactions. A rise in energy ex-

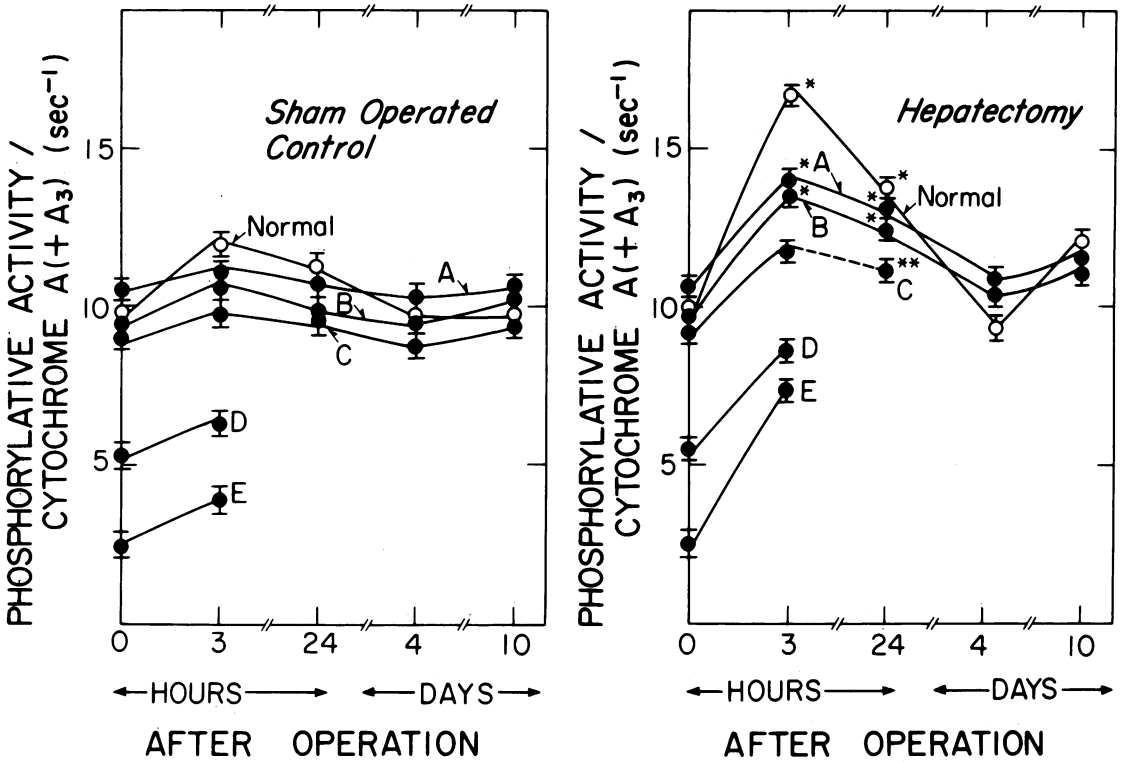

FIG. 4. Changes in phosphorylative activity per unit of cytochrome a(+a_3) of mitochondria from the remnant liver in five types of diabetic rats following partial hepatectomy. *Significantly higher than the corresponding sham operated normal controls. **Only 2 animals were studied.

penditure of the cells would result in a decrease of the energy charge, unless a concomitant increase in the rate of phosphorylation of ADP to ATP occurs.

After partial hepatectomy of groups A and B having no more than 28% inhibition in portal vein insulin response to glucose load, the energy charge levels of the remnant liver decreased slightly and could be restored rapidly toward normal levels, while after partial hepatectomy of groups C, D and E having more than 45% inhibition, the energy charge fell remarkably and could not be restored. The mitochondria obtained from the remnant liver of groups A and B exhibited a rise in the capacity to produce ATP which exceeded significantly the values of sham operated normal controls at 3–24 hours. On the contrary, an enhancement of mitochondrial phosphorylative activity did not reach the values of sham operated normal controls in groups C, D and E.

Such in vitro studies of isolated mitochondria might not, however, be directly indicative of the mitochondrial activity in situ, though the energy charge measured in the liver tissue can indicate the actual state of the tissue at a given time. Considering the recent observation[8] that insulin induces an enhancement in mitochondrial oxidative phosphorylation with a concomitant rise in the energy charge of perfused guinea pig liver, it is tempting to speculate that an enhancement in mitochondrial oxidative phosphorylation occurs as a result of conformational or configurational changes in one or all respiratory elements which are acquired in vivo and are not altered during the isolation and assay procedure. Thus, these results indicate that, despite the marked rise in the energy-utilizing reactions in early regeneration processes, the delicate balance between energy-utilization and energy-generation can subsequently be restored to normal by an enhancement of ATP generating reactions in groups A and B, but can not in groups C, D and E.

The possibility that an enhancement of liver mitochondrial function following partial hepatectomy in groups C, D and E is inhibited by the toxic effect of alloxan[24] may not be excluded completely. However, it has been found that the intraportal administration of insulin restores the oxidative and phosphorylative activities in the liver mitochondria of severely diabetic rats treated with alloxan.[19] Further evidence has accumulated,[8] indicating that an enhancement in mitochondrial oxidative phosphorylation of liver is induced by an elevated level of insulin available to hepatocytes. Consequently, it may be suggested that one possible rate limiting factor for an enhancement of liver mitochondrial phosphorylative activity in early regenerating processes may be availability of portal vein insulin for respiratory assemblies of liver mitochondria, and that the more severe the impairement in insulin secretion from the pancreas, the lower the potential functional capacity of liver. In mild diabetes the liver may be able to regenerate quickly after partial hepatectomy, by using a large quantity of available energy formed as a result of

TABLE 3. *Changes of Mitochondrial Oxidative Phosphorylation of the Remnant Liver in Five Types of Diabetic Rats following Partial Hepatectomy*

Oxidative Phosphorylation	Groups	Before Operation	3 hr Postop		24 hr Postop		4 Days Postop		10 Days Postop	
			Sham	Hepatectomy	Sham	Hepatectomy	Sham	Hepatectomy	Sham	Hepatectomy
RCR	Normal	5.3 ± 0.1 (6)	6.8 ± 1.1 (4)	7.9 ± 0.9 (6)	6.2 ± 0.7 (4)	6.9 ± 1.2 (6)	5.4 ± 0.7 (4)	4.8 ± 0.6 (6)	5.4 ± 0.3 (4)	5.6 ± 0.9 (4)
	A	4.6 ± 0.2 (6)	5.0 ± 0.5 (4)	5.1 ± 0.3 (6)	4.3 ± 0.7 (4)	4.9 ± 0.4 (4)	4.8 ± 0.6 (4)	4.0 ± 0.3 (4)	4.7 ± 0.5 (4)	4.8 ± 0.2 (4)
	B	4.0 ± 0.9 (6)	4.3 ± 1.1 (4)	5.6 ± 0.9 (6)	4.6 ± 0.5 (4)	4.4 ± 0.8 (6)	4.0 ± 0.4 (4)	3.9 ± 0.7 (4)	4.8 ± 0.3 (4)	4.6 ± 0.5 (4)
	C	3.7 ± 1.3 (6)	4.0 ± 0.9 (4)	4.7 ± 1.5 (6)	3.9 ± 0.8 (4)	4.6 ± 0.9 (2)	4.1 ± 0.3 (4)	—	4.3 ± 0.4 (4)	—
	D	3.2 ± 0.9* (6)	3.4 ± 0.7 (4)	4.2 ± 1.2 (6)	—	—	—	—	—	—
	E	1.9 ± 1.4* (5)	2.1 ± 0.4 (4)	3.1 ± 0.9 (5)	—	—	—	—	—	—
P/O	Normal	2.39 ± 0.08 (6)	2.16 ± 0.03 (4)	2.46 ± 0.07 (6)	2.46 ± 0.05 (4)	2.55 ± 0.13 (6)	2.32 ± 0.03 (4)	2.01 ± 0.27 (6)	2.38 ± 0.17 (4)	2.26 ± 0.03 (4)
	A	2.13 ± 0.16 (6)	1.97 ± 0.11 (4)	1.68 ± 0.10 (6)	1.96 ± 0.08 (4)	1.98 ± 0.04 (4)	2.02 ± 0.12 (4)	2.46 ± 0.267 (4)	2.15 ± 0.11 (4)	2.09 ± 0.08 (4)
	B	1.99 ± 0.18 (6)	1.98 ± 0.14 (4)	1.80 ± 0.33 (6)	2.01 ± 0.12 (4)	1.85 ± 0.08 (6)	2.00 ± 0.17 (4)	1.45 ± 0.09 (4)	2.04 ± 0.12 (4)	1.94 ± 0.09 (4)
	C	2.01 ± 0.27 (6)	1.81 ±0.31 (4)	1.89 ± 0.03 (6)	1.83 ± 0.37 (4)	1.88 ± 0.02 (2)	1.82 ± 0.12 (4)	—	2.10 ± 0.15 (4)	—
	D	2.16 ± 0.06 (6)	2.20 ± 0.52 (4)	1.88 ± 0.21 (6)	—	—	—	—	—	—
	E	1.14 ± 0.08* (5)	1.70 ± 0.02 (4)	1.65 ± 0.11 (5)	—	—	—	—	—	—
State 3/ cyt. a (sec^{-1})	Normal	2.08 ± 0.11 (6)	2.80 ± 0.03 (4)	3.48 ± 0.08† (6)	2.30 ± 0.04 (4)	2.91 ± 0.04 (6)	2.03 ± 0.06 (4)	2.75 ± 0.03 (6)	2.27 ± 0.08 (4)	2.63 ± 0.06 (4)
	A	2.45 ± 0.07 (6)	2.65 ± 0.06 (4)	4.22 ± 0.14† (6)	2.83 ± 0.08 (4)	3.35 ± 0.15† (4)	2.65 ± 0.12 (4)	2.25 ± 0.08 (4)	2.51 ± 0.07 (4)	2.83 ± 0.05 (4)
	B	2.49 ± 0.08 (6)	2.85 ± 0.09 (4)	3.85 ± 0.12† (6)	2.50 ± 0.08 (4)	3.40 ± 0.08† (6)	2.40 ± 0.04 (4)	2.63 ± 0.11 (4)	2.51 ± 0.10 (4)	2.95 ± 0.07 (4)
	C	2.41 ± 0.13 (6)	2.66 ± 0.07 (4)	2.79 ± 0.12 (6)	2.71 ± 0.06 (4)	2.80 ± 0.13 (2)	2.50 ± 0.11 (4)	—	2.33 ± 0.06 (4)	—
	D	1.27 ± 0.03* (6)	1.59 ± 0.05 (4)	2.25 ± 0.08 (6)	—	—	—	—	—	—
	E	1.15 ± 0.04* (5)	1.37 ± 0.08 (4)	2.21 ± 0.07 (5)	—	—	—	—	—	—
Phos./ cyt. a (sec^{-1})	Normal	9.9 ± 0.1 (6)	11.8 ± 0.2 (4)	16.7 ± 0.2† (6)	11.1 ± 0.1 (4)	13.7 ± 0.2† (6)	9.8 ± 0.1 (4)	9.3 ± 0.1 (6)	9.8 ± 0.2 (4)	12.0 ± 0.2 (4)
	A	10.5 ± 0.1 (6)	10.9 ± 0.1 (4)	14.0 ± 0.1† (6)	10.9 ± 0.1 (4)	13.0 ± 0.1† (4)	10.5 ± 0.1 (4)	11.0 ± 0.1 (4)	10.8 ± 0.1 (4)	11.6 ± 0.2 (4)
	B	9.9 ± 0.1 (6)	10.6 ± 0.1 (4)	13.7 ± 0.1† (6)	10.0 ± 0.2 (4)	12.3 ± 0.1† (6)	9.8 ± 0.2 (4)	10.5 ± 0.1 (4)	10.3 ± 0.1 (4)	11.0 ± 0.1 (4)
	C	8.9 ± 0.1* (6)	9.7 ± 0.1 (4)	11.5 ± 0.1 (6)	9.7 ± 0.1 (4)	11.1 ± 0.1 (2)	8.9 ± 0.1 (4)	—	9.5 ± 0.1 (4)	—
	D	5.5 ± 0.1* (6)	6.3 ± 0.2 (4)	8.7 ± 0.1 (6)	—	—	—	—	—	—
	E	2.6 ± 0.1* (5)	3.6 ± 0.1 (4)	7.4 ± 0.1 (5)	—	—	—	—	—	—

The results shown are mean values ± SEM.

Values in parenthesis indicate numbers of animals.

Oxygen consumption and phosphorylative activity were measured at 22° at pH 7.4 in medium containing 0.3 M mannitol, 0.01 M KCl, 0.004 M $MgCl_2$, 0.01 M Tris-HCl buffer, 0.005 M potassium phosphate buffer, 0.2 mM EDTA, and 230 μM ADP. Glutamate was added at a concentration of 4 mM. RCR, respiratory control ratio; P/O, moles of ATP formed per atom of oxygen consumed; State 3/cyt. a or phos./cyt. a, moles of oxygen utilized (or ATP formed) per second divided by the moles of cytochrome a($+a_3$).

* $P < 0.05$ compared to value of normal control group.

† Values significantly higher than the corresponding sham operated normal control group. ($P < 0.05$)

enhanced ADP-phosphorylating reactions by mitochondria, while the liver of severe diabetes can not adapt to hepatectomy in this way.

Mildly diabetic rats with relatively good insulin response to glucose load show a parabolic GTT pattern, while severely diabetic rats with highly inhibited insulin response to high blood glucose levels show a linear GTT pattern. The mortality rate by hepatectomy in the former is very low, while in the latter it is very high. Only the diabetic rats without ketonuria and with parabolic GTT pattern can tolerate the partial removal of the liver. The results of blood glucose response to glucose load in mildly and severely diabetic rats are consistent with two distinct patterns (parabolic and linear) of glucose intolerance classified in jaundiced patients[20] and animals[22] or patients with liver cancer.[21] Also, it has been found that the energy charge and mitochondrial oxidative phosphorylation of the liver is positively correlated with blood glucose and insulin levels in response to an oral glucose load.[9] Thus, glucose tolerance pattern in diabetes in human as well as rats is indicative not only of the severity of impaired insulin secretion but also of the possibility of the occurrence of a compensatory enhancement in mitochondrial oxidative phosphorylation, and may be useful in assessing the functional hepatic reserve.

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