EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON OXIDATIVE ENERGY METABOLISM IN RAT LIVER MITOCHONDRIA – A COMPARATIVE STUDY OF EARLY AND LATE EFFECTS

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ABSTRACT

The reports in the literature on effects of diabetes on mitochondrial energy-linked functions are conflicting. Hence we carried out systematic studies to evaluate the effects at the early and the late stages of the disease using STZ-diabetic rat as a model. At the end of one week, after induction of diabetes, respiration rates with glutamate and succinate as the substrates increased; respiration rates with other substrates e.g. β -hydroxybutyrate, pyruvate + malate and ascorbate + TMPD were not affected despite substantial decrease in the β -hydroxybutyrate dehydrogenase activity and cytochrome b and c+c₁ contents. Insulin treatment brought about increase in the cytochrome contents beyond control values. The ATPase activity was generally low in the diabetic animals and was not restored by insulin treatment.

At the end of one month, the respiratory activities with all the substrates were generally low. Insulin treatment either restored or stimulated the respiration rates beyond control values. The content of cytochromes was differentially affected in the diabetic animals, but insulin treatment caused significant increase beyond control levels. The pattern for ATPase activity was similar to the early effects.

At both the stages i.e. early and late stages of diabetes the mitochondria were tightly coupled. The ADP/O ratios were in normal expected ranges and the respiratory control ratios were comparable with the control groups. Insulin treatment resulted in apparent restoration of respiratory activity. However, the effects on the cytochromes and dehydrogenases activities were differential. Taken together the two observations would suggest that the mitochondria were not re-instated to normality despite apparent restoration of respiratory function.

KEY WORDS

STZ-diabetes, liver mitochondria, energy metabolism, cytochrome profile, ATPase.

INTRODUCTION

The basic defects in diabetes mellitus are impaired glucose metabolism and basement membrane alterations (1-3). Besides, mitochondrial oxidative energy metabolism also seems to be impaired in experimental diabetes (4-16). With respect to the mitochondrial respiratory activity one regulatory factor which is affected in diabetes is the pyruvate

dehydrogenase complex (17). More recent works suggests that mitochondrial diabetes may also affect the FoF₁ ATPase (18). Additionally, effects on glucokinase activity and on the citric acid cycle have also been described (19, 20). The mitochondria thus seem to be the direct targets of the diabetic state.

Effects of experimental diabetes on energy metabolism in the liver have been examined by several research workers (4-16), however, the reported effects are conflicting and contradictory (4-16). Thus, it has been reported that in the alloxan-diabetic animals the respiration rates and the P/O ratios decreased with the various substrates tested and that these defects were corrected by treatment with insulin (4, 5, 6). Beyer and Shamoan (7) and

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Mackerer *et al.* (8) reported increased respiration rates with normal P/O ratios. Brignone *et al.* and Bidetti *et al.* reported decreased coupled respiration along with impairment in the β -hydroxybutyrate dehydrogenase (BDH) activity in the streptozotocin-diabetic rats (9, 10). However, other investigators found no changes in the respiratory properties of liver mitochondria from streptozotocin-diabetic rats (11, 12) and the P/O ratios were found to be normal. (5, 11). It has also been reported that respiration rates and P/O ratios decreased 48 hr after alloxan treatment; the P/O ratios became normal 8 days after alloxan treatment or 4 hr after insulin treatment (6). Additionally, the time-dependent responses were found to be different in two strains of rats: Sprague-Dawley and Sherman (13). Interestingly, in the genetically-diabetic mice, respiration with glutamate and succinate was significantly elevated (14). Also, in obese hyperglycemic mice (ob/ob), the oxidative energy metabolism of the liver mitochondria has been reported to be significantly enhanced (15, 16). The apparent contradiction could possibly be attributed to several factors e.g. the nature of the diabetogen used, the dose employed, the route of administration and the time at which the effects were examined (4-16). Additionally, the responses could also vary depending on the strain of the animals used (13-16).

It is well recognized that diabetes progressively affects systems such as kidneys, retina, heart, peripheral and central nervous system and possibly liver and is thus systemic in nature (1). The major detectable symptoms, glucosuria and hyperglycemia can be controlled by treatment with insulin, but defects in tissues and systems in long standing diabetes do not get corrected by insulin treatment (1). Since the basic defect in diabetes is membrane alteration (2, 3), it is of interest to look for the possible membrane function alterations at various stages of the disease.

We therefore examined the early and late effects of streptozotocin-induced diabetes on the oxidative energy metabolism of rat liver mitochondria. Corrective actions of insulin were also examined. It is anticipated that examination of the diabetes-associated defects and efficacy of insulin treatment at early and late stages of the disease can help in getting better insights in the management of the maladies of the disease (1-3). Additionally, the problem of contradictory reports in the literature can also be addressed to (4-16). Results of these experiments are described here.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Company, USA. Sources of other fine chemicals are as described earlier (21-22).

Animals

Male albino rats of Wistar strain weighing between 200-230 g were used. The animals were fasted overnight and were made diabetic by injecting STZ intraperitoneally (i.p.) at a dose of 65 mg/Kg body weight as described earlier (23).

The diabetic state was ascertained in terms of the loss of body weight, polyuria, polyphagia and hyperglycemia (23, 24). The mortality ranged from 5 to 10%.

It has been shown earlier that a stable diabetic state is established only 2 weeks after the treatment with the diabetogen (17). Hence experiments were carried out at the end of one week or one month after induction of diabetes; these are taken to represent the early and the late stage of the disease (17).

In the one week group, the diabetic animals received NPH insulin (The Boots Company, Mumbai) at a dose of 0.5-0.6 units/ 100 g body weight twice daily (9 AM and 4 PM) starting from the third day of STZ administration. In the one month group, the diabetic animals received similar insulin treatment for 7 days starting from the third week of STZ administration (17).

Urinary and blood sugar levels were monitored as described earlier (23, 24).

The animals were killed by decapitation 18 hr. after the last injection of insulin.

The experimental protocol was approved by the Institutional Ethical Committee.

Isolation of mitochondria and study of oxidative phosphorylation

Liver mitochondria were isolated from 10 % (w/v) tissue homogenates according to the procedures described earlier (21, 22), washed once and suspended in the isolation medium (0.25 M sucrose) at the final concentration of approximately 40 mg protein / ml.

Measurements of oxidative phosphorylation were carried out in a Gilson oxygraph model KM (Gilson Medical Electronics, Middleton, WIS) using a Clark

type oxygen electrode as described earlier (21, 22) at 25°C in a total volume of 1.3 ml. The assay system contained: 225 mM sucrose, 4 mM MgCl₂, 10 mM potassium phosphate buffer and 10 mM tris-HCl buffer, all at pH 7.4. Substrate concentrations were : 10 mM; concentrations of malate and N,N,N',N', tetramethyl-*p*-phenylene diamine (TMPD) were 1 mM and 0.1 mM respectively. 1 µM rotenone was used with succinate and ascorbate + TMPD experiments (21, 22).

Calculations of the state 3 and state 4 respiration rates (obtained in the presence of added ADP and after its depletion, respectively) and of ADP-phosphorylation rates were as described earlier (21, 22).

Enzyme assays

The basal and Mg²⁺ and/or DNP- stimulated ATPase activities were determined as described previously (22). Measurements of glutamate dehydrogenase (GDH), β-hydroxybutyrate dehydrogenase (BDH) and succinate dehydrogenase (SDH) activities were according to the methods previously described (22).

Estimation of cytochrome content

Mitochondrial cytochromes contents were determined spectrophotometrically from the difference spectra of mitochondria after solubilization with triton X-100 (20, 21).

Protein estimation was according to Lowry *et al.* with bovine serum albumin used as the

standard (25).

Results are given as mean ± SEM. Statistical evaluation of the data was by students' t-test.

RESULTS

General

Administration of STZ resulted in loss of body weight, glucosuria and hyperglycemia (Table 1 and 2). The magnitude of the effects was always higher for the one month group. Insulin treatment completely restored the body weights in the one week group; in the one month group there was only partial restoration (Table 1). Insulin treatment completely cured polyuria and glucosuria in both the groups (Table 2). The serum glucose contents were only partially restored, and especially in the one week group the value was almost double the control (Table 2).

Effects on oxidative phosphorylation

Early effects

In the one week diabetic group, the state 3 respiration rates (ADP present) did not change for glutamate, β-hydroxybutyrate or ascorbate + TMPD. However, for succinate an increase of 23 % was noted. By contrast, the rate decreased for pyruvate + malate by 11 %. Insulin treatment stimulated glutamate oxidation rate while restoring that with pyruvate + malate; succinate oxidation rate was not corrected and remained elevated. The changes in the state 4 respiration rate (after depletion of added ADP) were marginal and the respiratory

Table 1. Effect of streptozotocin diabetes on body and liver weight

Duration	Animals	Body weight, g	Liver weight	
			g	% of Body weight
One week	Control (20)	238.5 ± 5.8	11.3 ± 0.3	4.7 ± 0.1
	Diabetic (20)	212.4 ± 2.0**	9.7 ± 0.2**	4.6 ± 0.1
	Diabetic + Insulin (20)	246.4 ± 4.6 ^b	13.5 ± 0.4 ^b	5.5 ± 0.2 ^{a,b}
One month	Control (30)	381.7 ± 15.8	15.8 ± 0.3	4.2 ± 0.1
	Diabetic (20)	231.1 ± 6.8**	9.9 ± 0.3**	4.3 ± 0.1
	Diabetic + Insulin (16)	263.9 ± 7.9 ^a	14.1 ± 0.8 ^b	5.4 ± 0.3 ^{a,a}
Results are given as mean ± SEM of the number of observations indicated in the parentheses. *, p < 0.002 and **, p < 0.001 compared with the corresponding control. ^a , p < 0.01 and ^b , p < 0.001 compared with the corresponding diabetic.				

Table 2. Effect of streptozotocin diabetes on urinary and serum glucose levels in the rat

Duration	Animals	Urine volume (ml)	Urinary glucose		Serum glucose (mM)
			mg/ml	g/24 h	
One week	Control (24)	1.5 ± 0.1	N.D.	-	6.1 ± 0.2
	Diabetic (24)	49.3 ± 5.5**	81.7 ± 1.1**	4.2 ± 0.1	24.9 ± 1.0**
	Diabetic + Insulin (20)	1.4 ± 0.2 ^a	N.D.	-	11.2 ± 0.6** ^a
One month	Control (20)	2.4 ± 0.3	N.D.	-	6.1 ± 0.2
	Diabetic (30)	102.2 ± 5.1**	83.1 ± 1.1**	8.5 ± 0.4	25.1 ± 0.6**
	Diabetic + Insulin (16)	1.5 ± 0.1** ^a	N.D.	-	8.7 ± 0.3** ^a

Results are given as mean ± SEM of the number of observations indicated in the parentheses.
^{*}, p < 0.01 and ^{**}, p < 0.001 compared with the corresponding control.
^a, p < 0.001 compared with the corresponding diabetic.
N.D. Not detectable.

control ratios (state 3 respiration rate/ state 4 respiration rate; data not shown) were generally high and in the expected ranges for the substrates employed (21, 22, 26). The ADP/O ratios were also in the normal expected ranges (21, 22, 26). Thus, the diabetic state did not cause any uncoupling of the mitochondria (Table 3). In the diabetic animals the contents of cytochromes b and c + c₁ decreased by 20 and 16 %. Insulin treatment restored c + c₁ content; those of b and aa₃ increased by 17 and 33 % beyond control (Table 5).

Of the primary dehydrogenases, GDH decreased by 15%; decrease in BDH was dramatically high (53% decrease) despite the increase in the state 3 respiratory activity noted above (Table 3). BDH decreased further in spite of the insulin treatment (61 % decrease). The SDH activity decreased by 50 % in the diabetics and was only partially restored after insulin treatment (Fig.1).

The basal ATPase activity (both Mg²⁺ and DNP absent) was low in the diabetics while the DNP and Mg²⁺+ DNP-stimulated ATPase activities were somewhat higher; these were restored by insulin treatment (Table 6).

Late effects

By the end of one month when the chronic diabetic state was established (17), the state 3 respiration rate decreased from 20-30 % for the different

substrates; no change was seen for ascorbate + TMPD (Table 4). The insulin treatment restored to normality the respiration rates with β-hydroxybutyrate, pyruvate + malate and succinate. The rate with glutamate increased by 27 % as in the early state of the disease (e.g. see Table 3). By contrast, a 20% decreased was noted for ascorbate + TMPD. The state 4 respiration rates were somewhat higher for the NAD⁺-linked substrates and succinate in the diabetics but the RCR values were always very high (data not shown). The ADP/O ratios were also in the expected theoretical range. Therefore, even in the late stage of the disease the mitochondria were well coupled. The ADP phosphorylation rates correlated well with the observed changes in the respiration rates (Table 4). Insulin treatment resulted in improved values for the respiratory control ratios and thus the mitochondria appeared to be even more tightly coupled.

The content of cytochrome aa₃ increased in the diabetics (25 % increase) while that of b decreased by 13 % (Table 5). Treatment with insulin restored the content of b, but those of aa₃ and c + c₁ increased by 68 and 61 % beyond control (Table 5).

The BDH activity decreased very significantly in the diabetics (80 % decrease); insulin treatment could not restore this activity. Other dehydrogenase activities were not affected in the diabetics and insulin treatment was without any effect (Fig.1).

Table 3. Early effects of streptozotocin diabetes on oxidative energy metabolism in rat liver mitochondria

Substrate Treatment	ADP/O ratio	Respiration rate (n mole O ₂ / min / mg protein)		ADP phosphorylation rate (n mole / min / mg protein)
		+ ADP	- ADP	
Glutamate				
Control (20)	2.84 ± 0.1	36.9 ± 1.2	1.0 ± 0.1	211.6 ± 9.3
Diabetic (18)	3.06 ± 0.1	36.9 ± 1.2	0.7 ± 0.1*	224.8 ± 8.9
Diabetic + Insulin (22)	2.81 ± 0.1	42.6 ± 2.0 ^a	1.1 ± 0.2 ^b	235.7 ± 13.4
β hydroxybutyrate				
Control (24)	2.86 ± 0.1	32.2 ± 1.8	1.0 ± 0.2	181.3 ± 9.2
Diabetic (18)	3.08 ± 0.1	33.0 ± 1.4	0.5 ± 0.1*	205.5 ± 9.8
Diabetic + Insulin (24)	2.74 ± 0.1	37.1 ± 2.1	1.5 ± 0.2 ^b	201.3 ± 10.4
Pyruvate+ Malate				
Control (30)	3.01 ± 0.1	28.9 ± 1.0	0.9 ± 0.2	170.9 ± 7.8
Diabetic (20)	3.08 ± 0.2	25.8 ± 0.8*	0.8 ± 0.1	160.0 ± 9.8
Diabetic + Insulin (22)	3.14 ± 0.1	29.9 ± 1.6 ^a	1.2 ± 0.2	180.8 ± 9.9
Succinate				
Control (24)	1.85 ± 0.1	73.1 ± 1.9	2.0 ± 0.3	270.8 ± 9.9
Diabetic (20)	1.91 ± 0.1	89.9 ± 4.2**	3.8 ± 0.4**	342.6 ± 12.4**
Diabetic + Insulin (22)	1.69 ± 0.1	82.9 ± 3.3**	5.8 ± 0.8**	281.5 ± 10.4 ^b
Ascorbate+TMPD				
Control (16)	0.35 ± 0.03	75.5 ± 5.2	25.7 ± 1.3	53.8 ± 3.8
Diabetic (24)	0.36 ± 0.02	67.8 ± 3.6	17.9 ± 0.7**	48.8 ± 2.9
Diabetic + Insulin (18)	0.34 ± 0.02	70.6 ± 7.3	21.1 ± 2.2	47.9 ± 5.0
Results are given as mean ± SEM of the number of observations indicated in the parentheses. *, p < 0.05 and **, p < 0.001 compared with the corresponding control. ^a , p < 0.05 and ^b , p < 0.001 compared with the corresponding diabetic.				

The ATPase activities were generally substantially low in the diabetic group ranging from 17-49 %; insulin treatment could not restore this change (Table 6).

DISCUSSION

The present studies were undertaken to establish a comprehensive picture of the early and late effects of the diabetic state on the energy metabolism of rat liver mitochondria for two reasons. Firstly, the earlier reports seemed to be

contradictory and conflicting (4-16). Secondly, it is very well established that in diabetes there is a progressive damage to the systems and tissues as the disease progresses (1). Therefore, understanding the underlying biochemical defects at the membrane level (e.g. with mitochondria as a model system), at the early and late stages of the disease can help in better understanding and management of the disease with insulin.

From the data presented, the clearly discernible effects of diabetes at the early stage were: a

Table 4. Late effects of streptozotocin diabetes on oxidative energy metabolism in rat liver mitochondria

Substrate Treatment	ADP/O ratio	Respiration rate (n mole O ₂ / min / mg protein)		ADP phosphorylation rate (n mole / min / mg protein)
		+ ADP	- ADP	
Glutamate				
Control (20)	2.96 ± 0.1	36.1 ± 1.9	1.0 ± 0.1	211.7 ± 9.3
Diabetic (20)	2.46 ± 0.1	29.0 ± 1.4*	1.5 ± 0.2	142.7 ± 8.9***
Diabetic + Insulin (24)	2.64 ± 0.1	45.9 ± 1.6** ^b	1.0 ± 0.1	242.4 ± 10.4** ^b
β hydroxybutyrate				
Control (20)	2.92 ± 0.1	34.2 ± 1.0	0.5 ± 0.1	199.7 ± 9.2
Diabetic (20)	2.66 ± 0.2	22.8 ± 1.6***	1.4 ± 0.2***	121.2 ± 9.4***
Diabetic + Insulin (24)	2.68 ± 0.1	34.8 ± 1.2 ^b	0.6 ± 0.1 ^b	185.6 ± 9.4 ^b
Pyruvate + Malate				
Control (20)	2.94 ± 0.1	32.2 ± 1.8	0.7 ± 0.1	189.4 ± 9.9
Diabetic (20)	2.65 ± 0.1	22.1 ± 1.1***	1.0 ± 0.2	117.0 ± 7.3***
Diabetic + Insulin (24)	2.79 ± 0.1	32.2 ± 1.2 ^b	1.2 ± 0.2*	174.7 ± 5.1 ^b
Succinate				
Control (22)	1.88 ± 0.1	68.1 ± 3.6	2.2 ± 0.3	260.1 ± 9.8
Diabetic (22)	2.03 ± 0.1	50.0 ± 3.0***	4.3 ± 0.5***	202.8 ± 10.2***
Diabetic + Insulin (26)	2.02 ± 0.1	72.7 ± 3.5 ^b	4.3 ± 0.5***	295.6 ± 19.1 ^b
Ascorbate + TMPD				
Control (14)	0.38 ± 0.03	76.6 ± 4.3	33.2 ± 1.2	57.8 ± 2.8
Diabetic (22)	0.36 ± 0.02	74.0 ± 6.7	26.4 ± 2.1**	53.3 ± 6.0
Diabetic + Insulin (24)	0.39 ± 0.02	61.2 ± 4.6*	30.0 ± 2.0	47.9 ± 5.0
Results are given as mean ± SEM of the number of observations indicated in the parentheses. *, p < 0.05; **, p < 0.01 and ***, p < 0.001 compared with the corresponding control. ^a , p < 0.05 and ^b , p < 0.001 compared with the corresponding diabetic.				

tendency towards decline of respiration with pyruvate + malate, a transient increase in the rate of succinate oxidation, impairment of BDH and SDH activities and decreased contents of b and c + c₁ cytochromes. Paradoxically, none of the changes correlated with the alterations in the respiration rates. Apparently, therefore, the respiratory potential of the mitochondria was more than sufficient in the initial stage of the disease to cope with the energy demand. Indeed, the ADP-phosphorylation rates were not changed significantly. In fact this value increased significantly for succinate. The ATP-synthesizing potential as judged in terms of the

ATPase activities also seemed to be adequate and generally unaffected. It is noteworthy that contrary to the earlier reports, (4-16) the mitochondria were well coupled with proper ADP/O ratios and respiratory control ratios.

Interesting feature of insulin therapy was that it stimulated respiration with glutamate but failed to regulate enhanced respiration with succinate. The increased contents of aa₃ and b cytochromes following insulin treatment could hardly be correlated with the observed respiration rates, nor was there any correlation with the ATPase activities.

Table 5. Effect of streptozotocin diabetes on cytochromes contents in the rat liver mitochondria

Duration	Animals	Cytochrome contents (p mole/ mg protein)		
		aa ₃	b	c + c ₁
One week	Control (16)	136.0 ± 5.0	216.0 ± 6.0	395.0 ± 6.0
	Diabetic (20)	129.0 ± 6.0**	173.0 ± 5.0***	331.0 ± 7.0***
	Diabetic + Insulin (12)	181.0 ± 4.0*** ^b	253.0 ± 8.0*** ^b	380.0 ± 11.0 ^b
One month	Control (14)	133.0 ± 7.0	211.0 ± 8.0	332.0 ± 12.0
	Diabetic (16)	164.0 ± 5.0**	183.0 ± 8.0*	262.0 ± 16.0**
	Diabetic + Insulin (10)	223.0 ± 4.0*** ^b	217.0 ± 12.0 ^a	535.0 ± 28.0*** ^b

Results are given as mean ± SEM of the number of observations indicated in the parentheses.
 *, p < 0.02; **, p < 0.002 and ***, p < 0.001 compared with the corresponding control.
^a, p < 0.05 and ^b, p < 0.001 compared with the corresponding diabetic.

Table 6. Effect of streptozotocin diabetes on ATPase activities in rat liver mitochondria

Duration	Animals	ATPase activity (μmole Pi liberated/hr/mg protein)			
		-Mg ²⁺ -DNP	+Mg ²⁺	+DNP	+Mg ²⁺ +DNP
One week	Control (12)	1.6 ± 0.4	1.7 ± 0.4	10.1 ± 0.5	9.1 ± 0.5
	Diabetic (16)	0.8 ± 0.2	1.5 ± 0.1	6.8 ± 0.6***	7.6 ± 0.5*
	Diabetic + Insulin (10)	0.9 ± 0.1	1.2 ± 0.1	6.3 ± 0.5***	5.6 ± 0.4*** ^a
One month	Control (12)	1.7 ± 0.2	1.7 ± 0.2	10.7 ± 0.3	10.3 ± 0.2
	Diabetic (12)	1.0 ± 0.2	1.5 ± 0.3	13.3 ± 0.3***	11.7 ± 0.4**
	Diabetic + Insulin (12)	1.4 ± 0.1***	1.7 ± 0.3	10.3 ± 0.6 ^b	9.7 ± 0.5 ^a

Results are given as mean ± SEM of the number of observations indicated in the parentheses.
 *, p < 0.05; **, p < 0.01 and ***, p < 0.001 compared with the corresponding control.
^a, p < 0.01 and ^b, p < 0.001 compared with the corresponding diabetic.

A clearer picture emerged at the end of one month when the chronic diabetic states was established (17). Thus the state 3 respiration rates decreased uniformly with all the substrates except ascorbate + TMPD. These were restored to normality by insulin treatment; with glutamate there was usual over stimulation. By contrast, the rate for ascorbate + TMPD decreased.

The decreased respiration rates in the diabetics were now correlated table with the decreased contents of cytochromes b and c+c₁, but increased aa₃ content vis a vis decreased respiration rate with ascorbate + TMPD was paradoxical.

It is possible that the increased aa₃ content may represent an attempt to increase the oxidative

potential. Insulin treatment caused an overall increase in cytochromes of all classes which ranged from 20 to 100 % over the diabetic values. Clearly this was much in excess than the observed increase in the respiration rates; especially the respiration rate with ascorbate + TMPD actually *decreased*. The ATP-synthesis rates followed pattern compatible with respiration rates: decrease in diabetics, restoration to normality with insulin treatment. Once again the ADP/O ratios and respiratory control indexes were in the normal expected ranges (21, 22, 26).

The BDH activity served as a clear pointer to the diabetic state; the significant decrease in this activity could not be corrected by insulin. Ketoacidosis in diabetes is well recognized (12,

Fig. 1. Effect of STZ diabetes and treatment with insulin on dehydrogenase activities in rat liver mitochondria. The results are given as mean $\pm$ SEM of 6-8 independent observations.

□, Control; ▨, Diabetic and
▩, +Insulin treated diabetic.

13).

The impaired ATPase activities in the diabetics were hardly corrected by insulin treatment; however this did not affect the ATP-synthesis rates.

It is thus clear from our present studies that the early and the late effects of the diabetic state on hepatic mitochondrial oxidative energy metabolism are quite different. It should also be emphasized that despite the restoration to normality of respiration rate after insulin treatment, these mitochondria differed significantly from the normal ones composition wise i.e. with respect to the

contents of the dehydrogenases, cytochromes and the ATPase activities. In other words the mitochondria were never re-instated to their normal physiologic state. Therefore it would certainly seem that all the maladies of diabetes can not be corrected by insulin therapy (1).

In related studies we have noted that the diabetic state and insulin treatment also affects differentially the functions of the kidney and the heart mitochondria; effects of STZ-diabetes were diverse compared to those reported here for the liver mitochondria (Unpublished work).

In conclusion, our present studies have provided for the first time a comprehensive and in depth picture of the early and late effects of the diabetic state on the hepatic mitochondrial energy metabolism and thus tried to resolve the earlier contradictory reports (4-16). Specifically, we have shown that at no stage were the mitochondria uncoupled; earlier reports may possibly relate to the toxic effects of alloxan accumulation (27). Besides, we have provided for the first time data on the compositional changes in the mitochondria with respect to the dehydrogenase activities, cytochromes contents and ATPase. However, since no correlation in general could be sought between the respiration rates and the compositional changes referred to above, it may be speculated that insulin may also influence the rate of electron transport *per se*. The precise mechanism by which insulin might exert this control is, however, not clear at this stage.

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