Streptozotocin-Induced Diabetes in the Chinese Hamster

Biochemical and Endocrine Disorders

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Summary. Streptozotocin treatment (125 mg/kg) in the Chinese hamster induced hyperglycaemia, hypoinsulinaemia, hyperglucagonaemia and changes in body, liver, pancreas, stomach, kidney and adipose tissue weights. The pancreatic reserves of insulin and glucagon in the diabetic animals were low, but stomach glucagon high. These animals showed high levels of phosphoenolpyruvate carboxykinase and low levels of glucokinase, hexokinase, isocitrate dehydrogenase and malic enzyme, but normal levels of pyruvate kinase in the liver. Increases in lactate dehydrogenase subunit B and isozymes 2, 3 and 4 were also observed in the liver, but not in the epididymal fat pad, of the diabetic animals. N-Acetyl- β -D-glucosaminidase was elevated in plasma, liver and heart, but not in the kidney of the treated animals. Renal α -galactosidase and β -glucosidase were depressed, whereas β -galactosidase and α -glucosidase remained essentially normal. These features indicated that there were considerable differences between the biochemical disorders associated with streptozotocin-diabetes in the Chinese hamster and the published observations in the rat.

Key words: Streptozotocin, Chinese hamster, glucagon, glycosidases, lactate dehydrogenase isozyme, insulin.

effect on islet A₂-cells, however, appeared to vary between species. For instance, streptozotocin was shown to induce occasional necrosis in A₂-cells of the Chinese hamster [2] and the rabbit [3], whereas it caused no change in pancreatic glucagon content in the rat [4] and led to predominance of A₂-cells in the guinea pig islets [5]. In view of the proposed involvement of pancreatic glucagon in the biochemical manifestations of diabetes [6], it is possible that related variations might be found in streptozotocin-induced diabetes among different species. Streptozotocin-diabetes in the rat has been studied extensively and its biochemical disorders include reciprocal changes in hepatic gluconeogenic and glycolytic enzymes [7], shift in adipose lactate dehydrogenase (LDH) (L-lactate: NAD oxidoreductase, EC 1.1.1.27) isozyme pattern [8, 9] and alterations of some β -glycosidases in the liver, kidney and plasma [10–12]. Knowledge about the properties of these biochemical entities in the streptozotocin-diabetic Chinese hamster, which in contrast to the diabetic rat develops necrosis of A₂-cells, is lacking. This study was thus undertaken to determine if dissimilar biochemical disorders occur in the streptozotocin-diabetic Chinese hamster as compared to those reported in the streptozotocin-induced diabetic rat.

Materials and Methods

Animals

In recent years, streptozotocin has been the most widely used agent to induce diabetes in laboratory animals. Its diabetogenicity arises from its cytotoxic effect on pancreatic islet B-cells and B-cell necrosis has been consistently demonstrated in all species of animals rendered diabetic by streptozotocin [1]. Its

Male Chinese hamsters, 4 to 7 months old, were selected from the Upjohn colony M line, which was a highly inbred non-diabetic line and had produced consistently aglycosuric offspring. After 20 h fasting the control animals were injected intraperitoneally with 10 ml/kg of 0.15 mol/l NaCl, adjusted to pH

4.0 with 0.05 mol/l citric acid. The experimental animals received 125 mg/kg streptozotocin dissolved in the same acidified saline solution at 12.5 mg/ml. They were allowed food and water ad libitum and sacrificed on the morning of the 14th or 42nd day.

Blood Samples

The animals were bled from the orbital sinus and blood was collected into small test tubes containing 500 U Trasylol® and 0.6 mg Na₂ EDTA [(ethylenedinitrilo) tetracetic acid, disodium salt] per 0.5 ml of blood. A small portion was removed for sugar determination by the ferricyanide method on an Autoanalyzer [13], and plasma was collected by sedimentation at 4° C. N-Acetyl- β -D-glucosaminidase was measured and the remaining plasma sample was kept at -20° C for insulin and glucagon measurements [14, 15].

Liver and Adipose Tissue Samples

After exsanguination and decapitation, the tissues were removed in the following order: liver, epididymal fat pad (epididymis + adipose tissue), heart, kidney, pancreas and stomach. The epididymal fat pad and a large portion of the liver were rinsed in cold saline, blotted, weighed on an analytical balance and ground in a Potter-Elvehjem homogenizer with a Teflon pestle in 2 volumes (tissue weight) of buffer containing 20 mmol/l tris·HCl, 0.15 mol/l KCl, 2 mmol/l EDTA, 0.5 mmol/l dithiothreitol, pH 8.0. After centrifugation at $140,000 \times g$ for 1 h, the liver supernatant was assayed for glucokinase (ATP: D-glucose-6-phosphotransferase, EC 2.7.1.2), hexokinase (ATP: D-hexose-6-phosphotransferase, EC 2.7.1.1) and PEP carboxykinase (GTP: oxaloacetate carboxylase, EC 4.1.1.32) and the remains were frozen in small aliquots and assayed for other enzyme activities and LDH isozyme distribution in the next three days. All samples were frozen and thawed only once. The fat pad supernatant was frozen for LDH activity and isozyme pattern determinations.

Liver, Kidney and Heart Samples

The remaining portion of liver, kidney and heart were rinsed in saline, blotted, weighed and homogenized in a Polytron® homogenizer at a setting of 5 for 30 sec in 9 volumes (tissue weight) of a chilled medium of 0.45 mol/l sucrose, 0.68 mmol/l EDTA, pH 7.0. After centrifugation at $12,000 \times g$ for 20 min, the supernatant was saved and the

pellet washed once with 5 ml of the same medium. The wash was discarded and the sediment was frozen in 9 volumes (tissue weight) of the medium in a dry ice-acetone bath, thawed at room temperature, repeated twice, and again homogenized. The supernatant and particulate fractions were kept at -20° C and assayed for various glycosidases within a week.

Pancreas and Stomach Samples

The pancreas was frozen immediately and the stomach was opened, rinsed in saline, blotted and frozen. Later, they were weighed and insulin and glucagon were extracted by homogenization in acid alcohol (750 ml of ethanol, 250 ml of water and 15 ml of conc. HCl). The homogenates were allowed to stand at 4° C for 24 to 48 h and centrifuged. The supernatants were neutralized with concentrated NH₄OH and the final supernatants were assayed for insulin and glucagon after appropriate dilutions.

Enzyme Assays

PEP carboxykinase was assayed by NaHCO₃-¹⁴C exchange method according to Wagle and Ashmore [16]. Glucokinase, hexokinase, pyruvate kinase (ATP: pyruvate phosphotransferase EC 2.7.1.40), LDH and protein concentration were determined as described previously [7]. Isocitrate dehydrogenase (Isocitrate: NADP oxidoreductase, EC 1.1.1.42) and malic enzyme (L-malate: NADP oxidoreductase EC 1.1.1.40) were assayed spectrophotometrically at 340 nm in reaction mixtures according to Khan with the omission of MTT-tetrazolium [3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide] and phenazine methosulfate [17].

Glycosidases were assayed with p-nitrophenyl derivatives as substrates [18]. p-Nitrophenyl glycosides were dissolved in buffer (0.1 mol/l Na₂HPO₄ adjusted to desired pH with 0.05 mol/l citric acid) at a concentration of 5.26 mmol/l. N-Acetyl- β -D-glucosaminidase and α - and β -galactosidase were assayed at pH 4.5, α -glucosidase at 6.5 and β -glucosidase at 5.5. The enzyme activities were determined from a linear time course in a reaction mixture containing 0.95 ml substrates and 0.05 ml appropriately diluted extracts. Reaction was terminated with 0.2 ml 300 g/l trichloroacetic acid and 0.2 ml 5 mol/l 2-amino-2-methylpropanediol was then added to the supernatant. The products were determined at 415 nm with p-nitro-phenol as standards.

All enzyme assays were run at 37° C.

Table 1. Body weight, tissue weight and protein concentrations in normal and streptozotocin-diabetic Chinese hamsters

Measurement	2 weeks after streptozotocin			6 weeks after streptozotocin		
	Control (6)	Strep (8)	P	Control (6)	Strep (6)	P
Total body wt. g	25.3 ±1.5	24.8 ±1.0	NS	30.1 ±0.2	26.5 ±0.5	<0.001
% Body wt:						
Liver	5.0 ±0.1	6.1 ±0.2	<0.005	5.0 ±0.1	6.2 ±0.2	<0.001
Pancreas	0.61±0.03	0.72±0.01	<0.05	0.54±0.02	0.81±0.08	<0.01
Stomach	0.89±0.02	1.14±0.04	<0.001	0.85±0.02	1.33±0.10	<0.001
Epididymal fat pad	4.1 ±0.4	3.0±0.2	<0.05	4.7 ±0.2	2.8 ±0.2	<0.001
Kidney	1.08±0.03	1.69±0.06	<0.001	0.96±0.02	2.20±0.32	<0.005
Heart	0.53±0.03	0.52±0.01	NS	0.44±0.01	0.49±0.02	NS
Protein conc. mg/g tissue:						
Liver						
Supernatant ^a	108±1	104±3	NS	129±2	136±3	NS
Precipitate	79±2	89±2	<0.001	81±3	93±4	<0.05
Kidney						
Supernatant	90±5	75±2	<0.05	92±2	85±3	<0.05
Precipitate	80±3	86±2	NS	90±3	88±2	NS
Heart						
Supernatant	43±2	42±1	NS	37±1	37±1	NS
Precipitate	119±5	106±4	NS	127±1	123±3	NS
Epidid. Fat Pad						
Supernatant	18±3	27±1	<0.05	13±1	18±1	<0.001

^a All supernatants were obtained after centrifugation at 12,000 × g

Table 2. Blood sugar, insulin and glucagon levels in normal and streptozotocin-diabetic Chinese hamsters^a

Measurement	Unit	2 weeks after streptozotocin			6 weeks after streptozotocin		
		Control (6)	Strep (8)	P	Control (6)	Strep (6)	P
Blood sugar	mg/100 ml	101±6	416±14	<0.001	98±6	423±43	<0.001
Plasma							
Insulin	μU/ml	40±14	0±0	<0.001	116±25	0±0	<0.001
Glucagon	pg/ml	109±19	212±9	<0.001	51±11	216±37	<0.01
Pancreas							
Insulin	U/g tissue	1.28 ±0.05	0.02 ±0.00	<0.001	0.70 ±0.03	0.03 ±0.005	<0.001
Glucagon	μg/g tissue	2.78 ±0.22	0.31 ±0.05	<0.001	3.39 ±0.27	0.61 ±0.10	<0.001
Stomach glucagon	μg/g tissue	0.012±0.001	0.031±0.003	<0.001	0.022±0.004	0.082±0.008	<0.001

^a Insulin and glucagon were measured by immunoassays using bovine insulin and porcine glucagon as standards

Table 3. Hepatic enzyme activities in normal and streptozotocin-diabetic Chinese hamsters

Enzymes	6 weeks after streptozotocin		
	Control (6)	Strep (6)	P
Phosphoenolpyruvate carboxykinase	nmole/min/mg		
	9.7±0.2	19.2±2.1	<0.001
Glucokinase	13.7±0.9	2.4±0.3	<0.001
Hexokinase	12.3±0.6	2.8±0.2	<0.001
Pyruvate kinase	456±23	385±26	NS
Isocitrate dehydrogenase	741±29	617±24	<0.01
Malic enzyme	161±24	59±10	<0.005

LDH Zymograms

Electrophoresis was run in 5% polyacrylamide slab gel at 300 V and 200 pulses/sec for 45 min [19], after a pre-running period of 2 h. The samples (0.01 ml) contained tissue extracts, 400 g/l sucrose and 10 mg/ml bromophenol blue in the proportion of 5:10:1 for the liver and 2:8:1 for the adipose tissue. The gels were stained for LDH [17] and the percent absorption of each isozyme band was quantitated on Quick Quant (Helena Lab.) after scan-

ning. The activities of LDH subunits A and B were then calculated [8].

Insulin and Glucagon Assays

Plasma and pancreas and stomach extracts were measured for insulin [14] and glucagon [15] by immunoassays. The lower limits of these assays were 4 μ U and 8 pg per sample for insulin and glucagon respectively. The standards for insulin were of bovine and for glucagon of porcine origins. The cross-reactivities of Chinese hamster insulin and glucagon to the antisera in our assays were similar to those of mouse and rat samples. Serial dilutions of Chinese hamster pancreatic extracts gave curves parallel to the bovine insulin or porcine glucagon standard curves.

Materials

125 I-Insulin was purchased from Nuclear International Corp.; 125 I-glucagon, Nuclear Medical Laboratory; beef insulin and porcine glucagon standards, Eli Lilly; glucagon antiserum 30K, University of Texas Southwestern Medical School. All substrates, cofactors and coupled enzymes in assays were purchased from Sigma.

All data were analyzed statistically with Student's t-test and are expressed as "mean $\pm$ SEM" in all tables.

Results

Tissues

Table 1 summarizes the comparison of body and tissue weights and tissue protein concentrations in control and experimental animals. The decrease in body weights was not observed at two weeks after streptozotocin administration, but was evident in the group studied six weeks after the injection. Changes observed at the 14th day included enlarged liver, pancreas, stomach and kidney and dystrophic epididymal fat pad and the changes were more pronounced on the 42nd day. The heart weight, however, remained normal in both groups of experimental animals. The protein concentrations in most tissues were also normal, with some exceptions. The liver $12,000 \times$ g particulate and adipose tissue $140,000 \times$ g supernatant fractions showed increased protein content and kidney supernatants had less proteins in the diabetic animals; the differences were observed at both 14th and 42nd days.

Insulin and Glucagon

The experimental animals were severely hyperglycaemic, with blood sugar levels over 400 mg/100 ml (Table 2). Plasma concentration of insulin was close to zero in these diabetic animals, whereas their plasma glucagon level was more than double that of the controls. Interestingly, pancreatic glucagon content was extremely low in these animals and their stomach glucagon level was three to four times that in the control animals (Table 2). The diabetic animals were also depleted of pancreatic insulin. These changes observed in the experimental animals were evident in both groups studied at 2 and 6 weeks after streptozotocin administration.

Day-to-day variation in insulin and glucagon immunoassays had been observed regularly in this laboratory and it contributed to the discrepancy in insulin and glucagon levels between animals in the control groups at 2 and 6 weeks after treatment. Assays on samples in a group, i.e. control and streptozotocin-injected animals either 2 or 6 weeks after injection, were always carried out in a single operation so that day-to-day variability could be avoided.

Hepatic Gluconeogenic and Glycolytic Enzyme Activities

At the 42nd day after streptozotocin injection, the animals showed twice as much hepatic PEP carboxykinase as those injected with saline (Table 3). On the other hand, their hepatic glucokinase and hexokinase activities were about 1/5 of those in the control animals. Surprisingly, the diabetic animals did not show significantly decreased levels of hepatic pyruvate kinase. Isocitrate dehydrogenase, however, was found to be slightly and significantly lowered and malic enzyme considerably depressed in the experimental animals (Table 3).

LDH Activity and Isozyme Pattern

LDH zymograms were scanned and analyzed in terms of isozyme distribution as shown in Table 4. The liver tissue of the diabetic animals showed a higher percentage of LDH-3 and -4 and less of LDH-5 than the controls. The specific activities of LDH subunits A and B were then calculated from these data and the tissue LDH activity [8]. The diabetic animals showed significantly higher hepatic LDH-B activity than the control animals. Studies on adipose tissue, however, showed similar LDH activity and isozyme patterns for both diabetic and non-diabetic animals. Again, concordant data were obtained in the two groups studied at different time periods.

Table 4. Tissue lactate dehydrogenase isozyme distribution in normal and streptozotocin-diabetic Chinese hamsters

Measurement		2 weeks after streptozotocin			6 weeks after streptozotocin		
		Control (6)	Strep (8)	P	Control (6)	Strep (6)	P
<i>Liver tissue</i>							
% total activity:	LDH-1	0	0	NS	0	0.9	NS
	LDH-2	0	0.7 ± 0.5	NS	1.0 ± 0.4	3.2 ± 0.8	<0.025
	LDH-3	0.8 ± 0.3	3.8 ± 1.3	NS	4.4 ± 0.3	9.4 ± 1.5	<0.01
	LDH-4	11.8 ± 0.7	16.9 ± 1.2	<0.025	14.6 ± 0.6	20.8 ± 0.9	<0.001
	LDH-5	87.4 ± 0.9	78.5 ± 2.8	<0.025	80.1 ± 1.1	65.7 ± 3.1	<0.001
μmole/min/mg protein:	LDH-A	1.97 ± 0.14	1.81 ± 0.13	NS	2.26 ± 0.17	1.78 ± 0.20	NS
	LDH-B	0.067 ± 0.007	0.13 ± 0.02	<0.01	0.16 ± 0.02	0.24 ± 0.03	<0.05
<i>Epididymal fat pad</i>							
% total activity:	LDH-1	7.4 ± 0.4	6.4 ± 0.5	NS	8.8 ± 0.4	8.4 ± 0.6	NS
	LDH-2	13.8 ± 0.4	13.7 ± 0.4	NS	14.8 ± 0.3	14.7 ± 0.6	NS
	LDH-3	19.2 ± 0.6	20.7 ± 0.4	NS	20.2 ± 0.4	20.1 ± 0.7	NS
	LDH-4	24.2 ± 0.9	25.7 ± 0.3	NS	22.5 ± 0.7	22.8 ± 1.0	NS
	LDH-5	35.3 ± 1.9	33.5 ± 1.0	NS	33.7 ± 1.0	34.0 ± 2.5	NS
μmole/min/mg protein:	LDH-A	0.44 ± 0.04	0.47 ± 0.02	NS	0.49 ± 0.04	0.53 ± 0.07	NS
	LDH-B	0.22 ± 0.01	0.24 ± 0.01	NS	0.27 ± 0.02	0.28 ± 0.03	NS

Table 5. N-acetyl-β-D-glucosaminidase in plasma, liver, heart and kidney in normal and streptozotocin-diabetic Chinese hamsters

Tissue		2 weeks after streptozotocin			6 weeks after streptozotocin		
		Control (6)	Strep (8)	P	Control (6)	Strep (6)	P
Plasma	U ^a /ml	63 ± 3	138 ± 3	<0.001	57 ± 2	114 ± 1	<0.001
Liver	U/g tissue	1343 ± 56	1960 ± 87	<0.001	1483 ± 31	2317 ± 189	<0.001
Supernatant ^b	U/mg prot.	4.3 ± 0.3	8.1 ± 0.8	<0.005	3.2 ± 0.2	6.9 ± 1.3	<0.025
Precipitate	U/mg prot.	11.1 ± 0.6	12.7 ± 0.4	<0.05	13.2 ± 0.3	14.8 ± 0.4	<0.01
Heart	U/g tissue	765 ± 39	802 ± 54	NS	636 ± 18	759 ± 41	<0.025
Supernatant	U/mg prot.	5.6 ± 0.2	6.0 ± 0.3	NS	5.0 ± 0.2	5.2 ± 0.3	NS
Precipitate	U/mg prot.	4.4 ± 0.1	5.1 ± 0.20	<0.025	3.6 ± 0.1	4.6 ± 0.3	<0.025
Kidney	U/g tissue	4220 ± 252	3411 ± 135	<0.025	4629 ± 138	4547 ± 109	NS
Supernatant	U/mg prot.	16.3 ± 0.8	13.5 ± 0.9	<0.05	11.9 ± 0.8	10.7 ± 0.6	NS
Precipitate	U/mg prot.	34.6 ± 0.9	30.2 ± 1.2	<0.025	39.6 ± 2.0	41.6 ± 1.8	NS

^a nmole substrate turnover per min^b Supernatants obtained after centrifugation at 12,000 × g**Table 6.** Renal galactosidases and glucosidases in normal and streptozotocin-diabetic Chinese hamsters

Enzyme		2 weeks after streptozotocin			6 weeks after streptozotocin		
		Control (6)	Strep (8)	P	Control (6)	Strep (6)	P
α-galactosidase	U ^a /g tissue	1537 ± 190	965 ± 57	<0.025	1001 ± 47	766 ± 37	<0.005
Supernatant ^b	U/mg prot.	9.9 ± 0.7	8.0 ± 0.6	<0.05	4.5 ± 0.5	3.0 ± 0.2	<0.025
Precipitate	U/mg prot.	6.3 ± 0.2	4.9 ± 0.3	<0.005	6.6 ± 0.4	5.8 ± 0.4	NS
β-galactosidase	U/g tissue	2125 ± 266	1850 ± 106	NS	1942 ± 57	1770 ± 74	NS
Supernatant	U/mg prot.	14.2 ± 1.2	12.8 ± 1.3	NS	8.2 ± 0.6	6.7 ± 0.5	NS
Precipitate	U/mg prot.	10.6 ± 0.6	10.8 ± 0.5	NS	13.2 ± 0.3	13.8 ± 0.8	NS
α-glucosidase	U/g tissue	147 ± 9	131 ± 3	NS	122 ± 5	108 ± 7	NS
Supernatant	U/mg prot.	1.1 ± 0.0	1.1 ± 0.0	NS	0.8 ± 0.1	0.7 ± 0.1	NS
Precipitate	U/mg prot.	0.6 ± 0.0	0.6 ± 0.0	NS	0.6 ± 0.0	0.5 ± 0.0	NS
β-glucosidase	U/g tissue	1111 ± 12	812 ± 34	<0.001	1004 ± 58	676 ± 60	<0.005
Supernatant	U/mg prot.	11.9 ± 0.5	10.1 ± 0.3	<0.025	10.5 ± 0.5	7.6 ± 0.5	<0.005
Precipitate	U/mg prot.	0.8 ± 0.1	0.6 ± 0.0	<0.005	0.4 ± 0.0	0.4 ± 0.0	NS

^a nmole substrate turnover per min^b Supernatants obtained after centrifugation at 12,000 × g

N-Acetyl- β -D-Glucosaminidase

The plasma level of N-acetyl- β -D-glucosaminidase in the diabetic animals was over twice as much as the control values (Table 5). Elevations of this enzyme in the experimental animals were also observed in liver and heart; in the liver excessive increase was more evident in the $12,000 \times g$ supernatant fraction, whereas in the heart the moderate increase was found only in the particulate fraction. The kidney, on the contrary, showed depressed activity of this enzyme in the streptozotocin-treated animals two weeks afterwards, and on the 42nd day its level returned to normal.

Renal Galactosidases and Glucosidases

The diabetic animals had a decreased level of α -galactosidase and β -glucosidase in the kidney and the decrease was more consistent in the supernatant fraction (Table 6). β -galactosidase and α -glucosidase, on the other hand, were not significantly different between the diabetic and control animals in both groups studied at 2 and 6 weeks after streptozotocin treatment. The renal content of these glycosidases followed this order: β -galactosidase > α -galactosidase > β -glucosidase > α -glucosidase.

Discussion

The purpose of this investigation was to determine if biochemical and endocrine disorders in the streptozotocin-diabetic Chinese hamster were similar to those reported in the rat [4, 7–12]. Our data demonstrated substantial differences between streptozotocin-diabetes in these two species of animals.

Our data on the depletion of both insulin and glucagon in the pancreas of the diabetic animal was consistent with the observation that streptozotocin induced necrosis of islet B- and A₂-cells in the Chinese hamster [2]. We have further demonstrated in the diabetic animal an excessive level of immunoreactive glucagon-like substances in the stomach, which certainly contributed to the elevated level of plasma glucagon as reported here. The increase in stomach immunoreactive glucagon most likely resulted from the depletion of pancreatic insulin and glucagon, rather than from the direct action of streptozotocin. Changes in the levels of pancreatic insulin and plasma and stomach glucagon, similar to those found in streptozotocin-diabetes, have also been observed in some highly inbred sublines of

hereditary diabetic Chinese hamster (unpublished observation). In view of the fact that some discordant features between streptozotocin-diabetes in the Chinese hamster and the rat had been reported to be glucagon- or cAMP-dependent, [8, 20, 21] elucidation of the biological and chemical characteristics of the extrapancreatic immunoreactive glucagon-like substances might give us clues to the cause of the species-dependent discrepancy. In the dog, at least, it has been shown that immuno-equivalent amounts of pancreatic and gastric fundic glucagons were not bioequivalent in their glycogenolytic and adenylate cyclase-stimulating activities [22].

The streptozotocin-diabetic rat had increased hepatic gluconeogenic and decreased glycolytic enzymes [7]. In streptozotocin-diabetic Chinese hamster liver, PEP carboxykinase, the most insulin-dependent gluconeogenic enzyme, was elevated and glucokinase, the most insulin-sensitive glycolytic enzyme, depressed. Hexokinase, measured in the $140,000 \times g$ liver supernatant, was also decreased in these animals. In rat liver cytoplasm, hexokinase existed mainly in type I form, which was reported to be insulin-insensitive [23]. Thus the observed depression in soluble hexokinase in streptozotocin-diabetic Chinese hamsters warrants investigation of the electrophoretic property of the hepatic glucose-phosphorylating enzymes in these animals. Likewise, a better understanding of the isozymic pattern of hepatic pyruvate kinase would also help us to explain the lack of significant depression of its activity in the diabetic Chinese hamster. Cytoplasmic malic enzyme and isocitrate dehydrogenase, which had been shown to be insulin-dependent [24, 25], were depressed in the diabetic Chinese hamster liver; changes in their levels correlated with the depletion of insulin in these animals.

Discrepancy between streptozotocin-induced diabetes in the Chinese hamster and the rat was also observed in the adipose LDH isozyme distribution. In the diabetic rat, a substantial increase in subunit B, as reflected in the elevations of LDH-1 and -2 and the reductions of LDH-3, -4 and -5, was found in the adipose tissue [8]. The diabetic Chinese hamster had normal LDH subunit activities and isozyme distribution in the epididymal fat pad, unlike the diabetic rat. On the other hand, increased LDH-B was observed in the liver tissue of the diabetic Chinese hamster, akin to the deviations found in the adipose tissue of the diabetic rat. It is speculated that the shift in LDH isozymes from A to B is to divert the carbon source to the gluconeogenic pathway in the event of increased lipid and glycogen

breakdowns and decreased pyruvate oxidation due to insulin deficiency, since LDH-B is inhibited by high concentration of pyruvate [26]. Such a shift would prevent the inordinate accumulation of tissue lactate. The data thus suggest that LDH isozyme pattern shifts in the diabetic rat adipose tissue and the diabetic Chinese hamster liver were caused by similar factor(s), the effects of which, however, were not manifested in the adipose tissue of the Chinese hamster. The nature of the causative factor(s) and the cause for the observed discrepancy remain unknown.

In the streptozotocin-diabetic rat, Fushimi and Tarui reported reductions in renal N-acetyl- β -D-glucosaminidase and normal levels of renal β -glucosidase and liver N-acetyl- β -D-glucosaminidase [10–12]. In their studies, they only measured enzyme activities in the $10,000 \times g$ supernatant of tissue extracts and claimed little activity remaining in the precipitates. We, however, observed substantial activities of these glycosidases in the residues and, therefore, measured them in both supernatant and particulate fractions. Our results indicated considerable discordance in the effects of streptozotocin-diabetes on the levels of these enzymes between the Chinese hamster and the rat. In the Chinese hamster rendered diabetic by streptozotocin, normal levels of renal N-acetyl- β -D-glucosaminidase and β -galactosidase, but reduced renal β -glucosidase and elevated liver N-acetyl- β -D-glucosaminidase, were found. The only concordant feature in glycosidase levels between the streptozotocin-diabetic rat and Chinese hamster was elevation in serum N-acetyl- β -D-glucosaminidase. In the diabetic Chinese hamster a moderate increase in heart N-acetyl- β -D-glucosaminidase was also observed. Furthermore, renal α -galactosidase was significantly decreased, whereas renal α -glucosidase remained essentially normal in these animals. Interestingly, in Chinese hamsters with hereditary diabetes, depression in α -galactosidase and a normal level of α -glucosidase were also found (our unpublished results). How significant a role the levels of these glycosidases play in glycoprotein turnover is unclear and its effect on the development of diabetic complications deserves further investigations.

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