Selective depletion of small basic non-glycosylated proteins in diabetes

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Degradative rates of small basic non-glycosylated proteins are preferentially enhanced in rat liver cytosol during severe streptozotocin-induced diabetes. Synthetic rates of these classes of proteins are not selectively enhanced in diabetes, so small basic non-glycosylated proteins should be depleted from liver cytosol as a consequence of this disease. To test this hypothesis, proteins were analysed from normal animals, from diabetic animals receiving insulin and from diabetic animals after insulin withdrawal for 3 days. The proteins were separated according to subunit molecular weight by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, according to isoelectric point by isoelectric focusing and according to carbohydrate content by affinity chromatography with concanavalin A linked to agarose. Severe uncontrolled diabetes is associated with the predicted depletion of small basic non-glycosylated proteins from liver cytosol. The preferential degradation and loss of these protein classes may be of considerable physiological importance to the animal.

Intracellular proteins are continually being degraded to their constituent amino acids and then replaced by new synthesis (Goldberg & Dice, 1974; Goldberg & St. John, 1976; Segal & Doyle, 1978; Dice & Goldberg, 1981). Alterations in the concentrations of specific enzymes or of total cellular protein can be achieved by changing rates of protein degradation, rates of protein synthesis or both (Goldberg & St. John, 1976; Dice & Goldberg, 1981). For example, in diabetes and starvation overall rates of liver protein synthesis are diminished and overall rates of proteolysis are increased (Garlick, 1980). Although the relative decline in protein synthesis and the increase in protein degradation in diabetes is rather small, the liver protein mass declines rapidly due to the rapid rate of protein turnover in liver (Garlick, 1980). Insulin deficiency is undoubtedly a major cause of the enhanced proteolysis in diabetes since this hormone has been shown to regulate protein catabolism in perfused livers (Mortimore & Schworer, 1980) and in isolated hepatocytes (Hopgood & Ballard, 1980). The situation is more complex in intact diabetic animals, however, since liver proteolysis is enhanced only for the first 2 days after diabetes but then declines below normal by the fourth day (Garlick, 1980).

We have previously examined whether degrada-

tive rates of any particular classes of cellular proteins are preferentially enhanced during the first 3 days of untreated diabetes (Dice *et al.*, 1978). In normal well-nourished animals polypeptides have heterogeneous half-lives, and protein structure appears to be an important determinant of protein degradative rates (Goldberg & Dice, 1974; Dice & Goldberg, 1981). Several correlations between the structure of native cytosolic proteins and their half-lives have been reported in liver and several other mammalian tissues. For example, high-molecular-weight proteins tend to be degraded more rapidly than small proteins (Dehlinger & Schimke, 1970; Dice *et al.*, 1973), acidic proteins tend to be degraded more rapidly than neutral or basic proteins (Dice & Goldberg, 1975), and glycoproteins are degraded more rapidly than non-glycosylated proteins (Kalish *et al.*, 1979). These relationships between protein structure and half-life are absent or markedly decreased in liver during the enhanced proteolysis associated with streptozotocin-induced diabetes (Dice *et al.*, 1978), most likely due to the preferential enhancement of degradation of protein classes that are normally long-lived. That is, during diabetes degradative rates of small basic non-glycosylated proteins tend to be selectively enhanced so that their half-lives approach those for the short-lived large acidic glycoproteins (Dice *et al.*, 1978; Dice & Goldberg, 1981; L. Bourret & J. F. Dice, unpublished work). Unless synthetic rates of small basic non-glycosylated proteins are also selectively

Abbreviation used: SDS, sodium dodecyl sulphate.

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enhanced in diabetes, these protein classes should be depleted from the cell.

Materials and methods

Animals

Albino male rats weighing 150–200 g (Charles River Laboratories, Wilmington, MA, U.S.A.) were maintained on Purina Lab Chow (Ralston Co., St. Louis, MO, U.S.A.) and water *ad libitum* for at least 5 days before their use in experiments.

Diabetes

Insulin-dependent diabetes was induced by intravenous injection of streptozotocin (Upjohn, Kalamazoo, MI, U.S.A.) (160 mg/kg body wt.) by the procedure described previously (Dice *et al.*, 1978). The severity of diabetes was monitored by measurement of body weight, urine glucose (Tes-tape; Eli Lilly, Indianapolis, IN, U.S.A.), and urine ketones (Ketostix; Ames Laboratories, Elkhart, IN, U.S.A.). Diabetic animals were maintained for at least 4 days with a single daily injection (at 17:00 h) of 2–4 units of NPH-U40 insulin (Eli Lilly, Indianapolis, IN, U.S.A.). By this procedure acute insulin withdrawal could be achieved and any immediate effects of the streptozotocin treatment avoided. Livers were removed from non-diabetic rats, diabetic rats receiving insulin, and diabetic rats deprived of insulin for 3 days. All rats were killed at 12:00–13:00 h to control for diurnal variations. At the time of hepatectomy blood glucose was determined (Dow Diagnostics, Indianapolis, IN, U.S.A.).

Protein synthesis measurements

A diabetic rat was maintained on insulin for 5 days. Insulin was then withdrawn for 3 days during which time the animal lost 24% of its body weight and developed slight ketoacidosis. This animal was injected intraperitoneally with 150 μ Ci of [14 C]-leucine (sp. radioactivity 309 mCi/mol), and a normal rat was injected with 750 μ Ci of [3 H]-leucine (sp. radioactivity 6 Ci/mmol; both isotopes were from Schwarz/Mann, Orangeburg, NY, U.S.A.). Both animals were injected at 09:00 h and 4 h later the animals were killed and the livers were mixed before homogenization and preparation of cytosolic proteins.

Preparation of cytosolic proteins

Before hepatectomy, the animals were anaesthetized with diethyl ether and the liver was perfused *in situ* with 250 ml of phosphate-buffered saline (72 mM- Na_2HPO_4 /28 mM- NaH_2PO_4 /26 mM- NaCl , pH 7.2) to remove blood. Ice-cold phosphate-buffered saline (2.5 vol., v/w) was added to the excised liver, which was then minced and homogenized with ten strokes of a Teflon pestle in a

Potter–Elvehjem homogenizer. Homogenates were first centrifuged for 10 min at 10 000 *g* and then the supernatant was centrifuged for 1 h at 100 000 *g* at 4°C. The supernatant from this spin (cytosol) was then dialysed against phosphate-buffered saline and frozen in small portions at –20°C. Samples were frozen and thawed only once before analysis.

SDS/polyacrylamide-gel electrophoresis and isoelectric focusing

Proteins were fractionated according to subunit molecular weight in 19 mm-diameter cylindrical gels for measurements of protein synthesis (Dice *et al.*, 1973). For staining analyses approximately equal amounts of each protein fraction (150 μ g) were applied to different lanes of 3 mm-thick slab gels in a Bio-Rad model 220 slab gel apparatus (Bio-Rad Laboratories, Richmond, CA, U.S.A.). Two different gel recipes were used for the slab gels. The first was a modification (Dice *et al.*, 1973) of the procedure of Weber & Osborn (1969), and the second was as described by Laemmli & Favre (1973). Isoelectric focusing of protein samples (150 μ g) was done in polyacrylamide gels as described by Bio-Rad Laboratories (1977) and in 110 ml columns as reported previously (Dice *et al.*, 1979).

Separation of glycoproteins from non-glycosylated proteins

Fractions enriched for glycoproteins or non-glycosylated proteins were obtained by passing cytosol through a column of concanavalin A linked to agarose (Glycosylex; Miles-Yeda, Elkhart, IN, U.S.A.) as described in detail elsewhere (Kalish *et al.*, 1979). Proteins that do not bind to the concanavalin A are enriched for non-glycosylated proteins, whereas proteins that bind but are then eluted with the competing sugar, α -methyl D-mannoside, are highly enriched for glycoproteins (Kalish *et al.*, 1979). Eluted fractions were dialysed extensively against phosphate-buffered saline, and protein content was determined by the method of Lowry *et al.* (1951).

Counting of radioactivity, gel staining and spectrophotometric measurements

Counting of ^3H and ^{14}C radioactivity in SDS/polyacrylamide gels, isoelectric focusing fractions and fractions from concanavalin A affinity chromatography was done as reported previously (Kalish *et al.*, 1979). SDS/polyacrylamide slab gels were stained with Amido Black (Dice *et al.*, 1973) or Coomassie Blue (Chrambach *et al.*, 1967). Isoelectric focusing gels were stained with Coomassie Blue R250 by method B of Vesterberg *et al.* (1977). Tracks from the SDS gels or the entire cylindrical isoelectric focusing gel were placed in a quartz cuvette and scanned with a Gilford model 250

spectrophotometer equipped with a gel scanning attachment (Gilford Instruments, Oberlin, OH, U.S.A.). Tracings of the gels were superimposed and the maximum absorbance of corresponding protein bands was compared for protein fractions from different animals.

Statistical calculations

To determine whether small or basic proteins tended to be depleted in diabetes, linear correlation coefficients were calculated between the absorbance ratios of protein bands (absence of insulin/presence of insulin) and their migration distances in SDS/polyacrylamide gels (Figs. 2 and 3) or isoelectric focusing gels (Fig. 4). The significance of the correlation coefficient was obtained from statistical tables (Owen, 1962). The significance of depletion of non-glycosylated proteins was calculated by using a two-tailed Student's *t* test.

Results

Animals

Streptozotocin-diabetic rats maintained with daily insulin injections gained weight at rates equal to or greater than the control animals. When insulin was withdrawn from the diabetic rats, glucosuria developed within 1 day and ketonuria developed by 3 days in some but not all animals. None of the diabetic animals gained weight after insulin withdrawal, but weight loss varied from 0 to 27% of the body weight. Blood glucose levels after 3 days of insulin withdrawal were also variable (332–534 mg/100 ml). Liver wet weights were slightly decreased in the diabetic animals not receiving insulin, and this decline coupled with the reduced body weight indicates a loss of liver mass after insulin withdrawal. The protein concentrations in liver were not affected by the insulin withdrawal, so loss of liver

protein roughly follows loss of liver wet weight. These results are summarized in Table 1.

Initial experiments established that diabetic rats receiving insulin and untreated normal rats had identical patterns of incorporation of radioactive leucine into proteins as separated by subunit size, net charge or carbohydrate content. Furthermore, the relative concentrations of proteins separated by these parameters were indistinguishable between normal animals and diabetic animals maintained with insulin. Therefore, some studies in the present paper compared diabetic animals after insulin withdrawal with diabetic animals receiving insulin, whereas other studies used normal untreated animals as controls.

Synthesis of liver proteins in diabetes

The relative rates of synthesis of different classes of liver proteins were compared in a normal rat and a diabetic rat after 3 days of insulin withdrawal. According to the labelling protocol described in the Materials and methods section, protein fractions with low $^3\text{H}/^{14}\text{C}$ ratios indicate enhanced synthesis after insulin withdrawal. Proteins were separated on the basis of subunit size, net charge or carbohydrate content, and ^3H and ^{14}C radioactivity was measured. Insulin withdrawal appears to have heterogeneous effects on synthesis of proteins of different molecular weights, since the variability in $^3\text{H}/^{14}\text{C}$ ratios is greater than when analogous experiments are done with two normal animals. The stippled region indicates two standard deviations from the mean $^3\text{H}/^{14}\text{C}$ ratios in such a control experiment. The fact that virtually all protein fractions have $^3\text{H}/^{14}\text{C}$ ratios higher than values for the control animals does not necessarily indicate that average rates of protein synthesis were decreased by diabetes. Decreased ^{14}C incorporation could reflect differences in amino acid transport or in amino acid pool sizes in diabetic

Table 1. Characteristics of normal and streptozotocin-diabetic rats

Rat	Rat no.	Blood glucose (mg/100 ml)	Liver weight (% of body wt.)	Body weight (g)	Weight change in last 3 days of experiment (%)
Normal	1	216	4.55	190	+10
	2	170	4.34	201	+12
	3	167	4.88	167	+10
	4	135	4.61	196	+13
	5	146	4.72	185	+11
Diabetic + insulin	1	241	5.05	194	+13
	2	179	5.16	224	+21
Diabetic—insulin	1	430	3.88	186	0
	2	510	4.12	165	—8
	3	332	3.86	133	—15
	4	534	3.95	147	—16
	5	490	3.75	127	—27

animals, and other methods are necessary to accurately compare rates of protein synthesis in animals in different metabolic states (Garlick, 1980).

Fig. 1. Relative synthetic rates of different classes of liver cytosolic proteins in normal and diabetic rats

A normal rat was injected with [^3H]leucine and a diabetic rat that had had its insulin withheld for 3 days was injected with [^{14}C]leucine as described in the Materials and methods section. Radioactive cytosolic proteins were separated by SDS/polyacrylamide-gel electrophoresis (Fig. 1a), isoelectric focusing (Fig. 1b) or affinity chromatography with concanavalin A linked to agarose (Fig. 1c) as described previously (Kalish *et al.*, 1979). In (a) arrows indicate the positions of protein standards determined as described by Dice *et al.* (1978). The stippled areas in (a) and (b) represent the averages ± 1 S.D. for $^3\text{H}/^{14}\text{C}$ ratios from experiments done with two normal animals. The error bars in (c) represent ± 1 S.D. from the average of three determinations using the same affinity column. The different experiments used different affinity columns but the same protein preparations. Abbreviations used: BSA, bovine serum albumin; OVAL, ovalbumin; LYSOZ, lysozyme. Key to (c): ▨, total; ▤, glycoproteins; ■, non-glycosylated proteins.

Although comparisons of absolute $^3\text{H}/^{14}\text{C}$ ratios between sets of animals are not valid, comparisons of $^3\text{H}/^{14}\text{C}$ ratios of proteins from the same set of animals do provide an indication of the relative rates of synthesis among proteins within that sample. That

Fig. 2. Depletion of small cytosolic proteins from rat liver during insulin withdrawal: SDS/polyacrylamide-gel electrophoresis by the Weber-Osborn method

Proteins were separated according to subunit molecular weight and stained in 1% Amido Black in 10% acetic acid. Destained gels were scanned spectrophotometrically, and the absorbance of protein bands was compared as described in the Materials and methods section. Arrows indicate the positions of protein standards run concurrently in a different track of the slab gel, with their molecular weights in parentheses. (a) shows the gel scans from an animal receiving insulin (Table 1, rat no. 2) and one that had its insulin withdrawn for 3 days (Table 1, rat no. 4). The continuous line in (b) shows the absorbance ratios from these scans and demonstrates a significant trend to deplete low-molecular-weight proteins after insulin withdrawal (correlation coefficient, $r = -0.703$; $P < 0.005$). The broken line in (b) shows a similar depletion of low-molecular-weight proteins in another rat after insulin withdrawal (Table 1, rat no. 5) when compared with a rat maintained on insulin (Table 1, rat no. 2) (correlation coefficient, $r = -0.648$; $P < 0.005$). The error bar in (b) represents the average ($1 \pm$ S.D. for ratios of protein peaks from four separate diabetic animals maintained with insulin. Abbreviations used: BSA, bovine serum albumin; OVAL, ovalbumin; CYT.C, cytochrome c.

is, if small proteins were preferentially synthesized during untreated diabetes, the $^3\text{H}/^{14}\text{C}$ ratios would be lower at greater migration distances. In fact, there is no significant correlation between $^3\text{H}/^{14}\text{C}$ ratios and migration distances in the gel. Note that the high $^3\text{H}/^{14}\text{C}$ ratio of proteins at mol.wt. 24 000 suggests that synthesis of these proteins is selectively decreased in diabetes. Fig. 1(b) shows that proteins with basic isoelectric points are not preferentially synthesized during untreated diabetes since there is no significant correlation between $^3\text{H}/^{14}\text{C}$ ratio and isoelectric point. Once again, the stippled region indicates two standard deviations from the mean $^3\text{H}/^{14}\text{C}$ ratio in an analogous experiment done with two normal rats. Fig. 1(c) shows results from two separate experiments that indicate that non-glycosylated proteins are not preferentially synthesized during diabetes.

Selective depletion of small proteins

Since small proteins are catabolized more rapidly but are not synthesized more rapidly in livers of diabetic animals, their concentrations should be decreased in this disease. Cytosolic proteins (approx. 150 μg) were separated by using a modification of the Weber–Osborn method of SDS/polyacrylamide-gel electrophoresis and the gels were stained with Amido Black and destained as described by Dice *et al.* (1973). We initially carried out several control experiments to establish the reliability of comparing scans of two different gels. First, cytosolic proteins from a normal rat were separated on different gels run concurrently. Next, cytosolic proteins from four normal rats were compared. Cytosolic proteins from four diabetic rats receiving insulin were also compared, and finally, proteins from two different diabetic rats receiving insulin were compared with those from two normal animals. The average variation of protein band absorbances was approx. 10% in each case. In no instance did we observe a statistically significant relationship between molecular size and depletion of proteins.

The spectrophotometric scan of proteins from a diabetic animal receiving insulin and one with its insulin withheld for 3 days are shown in Fig. 2(a). Approx. 25 peaks could be distinguished representing polypeptides ranging in molecular weight from 11 000 to 180 000. All major protein bands were evident in both insulin-treated and insulin-withdrawn animals. However, comparison of the absorbances of the peaks indicates that low-molecular-weight proteins tended to be depleted during insulin withdrawal (Fig. 2b). The broken line in Fig. 2(b) shows the results of a similar experiment comparing a different diabetic animal after insulin withdrawal with the same animal receiving insulin.

A similar analysis of protein subunit size in liver cytosol of normal and diabetic rats was conducted

using the slab gel method of Laemmli & Favre (1973). Once again, control experiments indicated expected deviations of $\pm 10\%$ in absorbance ratios of proteins from two normal animals. Fig. 3(a) indicates that the spectrophotometric scans were qualitatively similar to those using the modified Weber–Osborn system. However, the resolution of certain protein bands, especially in the high-molecular-weight range, was improved. The results plotted in Fig. 3(b) show absorbance ratios of protein bands from two different diabetic animals compared with two different normal animals. In each case, there tended to be preferential loss of smaller proteins. Significant depletion of low-molecular-weight proteins from liver cytosol was also evident when unstained gels were scanned at 280 nm and when gels were stained with Coomassie Blue.

In addition to the depletion of small proteins in diabetes, the accumulation of proteins in a high-molecular-weight fraction (the second data point at mol.wt. 150 000) is apparent with this gel system. Our previous results suggest that the degradation of proteins in this particular molecular-weight range may be markedly decreased in diabetes (see Fig. 1 of Dice *et al.*, 1978), but the identity of these polypeptides awaits further studies. It should be noted, however, that the depletion of small proteins relative to large proteins in diabetes is statistically significant whether or not the second data point is included in the analysis.

Selective depletion of basic proteins

Since degradation of basic proteins is preferentially enhanced in diabetes (Dice *et al.*, 1978), whereas their relative rates of synthesis are not increased (Fig. 1b), basic polypeptides should also tend to be depleted from liver cytosol of diabetic rats. Control experiments in which proteins from four different diabetic animals receiving insulin were compared indicated the deviation in absorbance ratios expected due to experimental error ($\pm 15\%$; Fig. 4b). Fig. 4 shows the spectrophotometric scan of isoelectric focusing gels for a diabetic animal whose insulin had been withdrawn for 3 days compared with a diabetic animal receiving insulin. As shown in Figs. 4(a) and 4(b), the basic proteins tended to be preferentially depleted after insulin withdrawal.

These results were qualitatively confirmed using isoelectric focusing in 110 ml columns rather than in polyacrylamide gels. In these experiments liver cytosolic proteins were separated into acidic (pI 4–7) and basic (pI 7–12) fractions and dialysed extensively against phosphate-buffered saline to remove ampholines and sucrose. The protein content of each fraction was determined by the method of Lowry *et al.* (1951). In separate experiments comparing two normal and three diabetic animals whose insulin had

Fig. 3. Depletion of small cytosolic proteins from rat liver during insulin withdrawal: SDS/polyacrylamide-gel electrophoresis by the Laemmli-Favre method

Proteins were separated on the basis of subunit molecular weight (Laemmli & Favre, 1973) and stained in 1% Amido Black in 10% acetic acid. Destained gels were scanned spectrophotometrically, and the absorbance of protein bands was compared as described in the Materials and methods section. In these experiments, the absorbance optimum for a new lot of Amido Black was found to be 600nm rather than 590nm. Also, initial experiments demonstrated that the variation in absorbance of protein peaks was indistinguishable for diabetic rats receiving insulin and for normal animals. Therefore, normal animals rather than diabetic animals receiving insulin were used as controls in these experiments. Arrows indicate the positions of protein standards run concurrently in a different track of the slab gel. (a) shows the gel scans from a normal animal (Table 1, rat no. 2) and a diabetic animal with its insulin withdrawn for 3 days (Table 1, rat no. 3). (b) shows the absorbance ratios from these scans and demonstrates a significant depletion of low-molecular-weight proteins in the diabetic animal ($\square$) (correlation coefficient, $r = -0.663$; $P < 0.005$). A second comparison of these two sets of liver proteins on a different day gave qualitatively similar results ($\bullet$) (correlation coefficient, $r = -0.557$; $P < 0.01$). A second diabetic

Fig. 4. Depletion of basic proteins from rat liver cytosol during insulin withdrawal: isoelectric focusing in polyacrylamide gels

Proteins from a diabetic animal receiving insulin (Table 1, rat no. 2) and one that had its insulin withdrawn for 3 days (Table 1, rat no. 4) were compared after isoelectric focusing as described in the Materials and methods section. The pH gradient was determined in a separate gel run concurrently. Protein standards of known isoelectric points were run in another gel, and their migration positions are indicated by arrows. The error bar in (b) represents the average ± 1 s.d. for ratios of protein peaks from four separate diabetic animals maintained with insulin. The correlation coefficient ($r = -0.676$; $P < 0.005$) indicates a significant trend to selectively deplete basic polypeptides. Abbreviations used: OVAL, ovalbumin; Hb, haemoglobin; CYT.C, cytochrome c.

animal (Table 1, rat no. 2) was compared with the same normal animal ($\circ$) (correlation coefficient, $r = -0.650$; $P < 0.005$), and with a different normal animal (Table 1, rat no. 1) (Δ) (correlation coefficient, $r = -0.639$; $P < 0.005$). All four correlation coefficients were still significant even if peak 2 was omitted from the analysis. Abbreviations used: β -GAL, β -galactosidase (mol.wt. 135 000); BSA, bovine serum albumin (mol.wt. 67 000); OVAL, ovalbumin (mol.wt. 45 000).

Table 2. Depletion of basic proteins from rat liver cytosol during insulin withdrawal: isoelectric focusing in preparative columns

Proteins from two normal and three diabetic animals were fractionated according to isoelectric point by the procedure described by Dice *et al.* (1979). Acidic (pI = 4–7) and basic (pI = 7–12) fractions were pooled, dialysed and protein concentrations were determined by the method of Lowry *et al.* (1951).

Rat	Rat no.	Cytosolic liver proteins (%)	
		Acidic	Basic
Normal	4	68	32
	5	65	35
Diabetic—insulin	3	82	18
	4	81	19
	5	77	23

Fig. 5. Depletion of non-glycosylated proteins from rat liver cytosol during insulin withdrawal: affinity chromatography with concanavalin A-agarose

Proteins from a diabetic animal receiving insulin (Table 1, rat no. 2) and one that had its insulin withdrawn for 3 days (Table 1, rat no. 4) were fractionated into glycoproteins and non-glycosylated proteins as described (Kalish *et al.*, 1979). Seven fractions (2ml each) were collected before and after the addition of the eluting sugar. Protein concentrations in each fraction were determined by the method of Lowry *et al.* (1951) after extensive dialysis. The values for the animal receiving insulin (●) are averages $\pm$ 1 S.D. for three separate experiments using different affinity columns. The values for the animal after insulin withdrawal (○) represent a single experiment. After insulin withdrawal, the proportion of glycoproteins was increased and the proportion of non-glycosylated proteins was decreased.

been withdrawn for 3 days, basic proteins comprised a smaller proportion of the total cytosolic protein (Table 2).

Selective depletion of non-glycosylated proteins

Among liver cytosolic proteins from normal rats, 3–6% bind to concanavalin A and are selectively eluted by competing sugar, α -methyl-D-mannoside (Kalish *et al.*, 1979). Our previous studies indicate that approx. 70% of the glycoproteins in cytosol bind to concanavalin A, so the separation is not pure. However, the proteins that do not bind to concanavalin A are enriched for non-glycosylated proteins, and the proteins that do bind are greatly enriched for glycoproteins (Kalish *et al.*, 1979).

Fig. 5 shows one typical experiment to illustrate the protocol. Proteins from a diabetic animal receiving insulin were compared with proteins from a diabetic rat that had its insulin withdrawn for 3 days. Equal amounts of protein were applied to two separate columns of concanavalin A-agarose and fractions were collected, dialysed and protein concentrations were determined (Lowry *et al.*, 1951). The concentration of non-glycosylated proteins in the cytosol is decreased relative to the concentration of glycoproteins.

Fig. 6. Depletion of non-glycosylated proteins from rat liver cytosol during untreated diabetes: affinity chromatography with concanavalin A-agarose

Protein fractionation was achieved as described in the legend to Fig. 5, except glycoprotein and non-glycosylated protein peaks were pooled before protein concentration measurements. The data points represent determinations made on different days and often with different affinity columns. The bars indicate the average values for the proportion of recovered protein as non-glycosylated proteins for experiments with five normal and two diabetic animals.

Similar experiments have been repeated for five normal animals and two diabetic animals that were deprived of insulin. Fig. 6 shows that the diabetic animals had decreased concentrations of non-glycosylated proteins. The individual points represent different experiments with the same protein preparation but with different affinity columns. Although only two diabetic rats were analysed, diabetes was associated with a statistically significant decrease in concentrations of non-glycosylated proteins ($P < 0.005$). This calculation was carried out by comparing the average percentage of non-glycosylated proteins from the five normal and the two diabetic animals with a two-tailed Student's t test. For reasons that are not clear, the diabetic animals showed greater variation in repeated experiments than did normal animals.

Discussion

The selective depletion of small basic non-glycosylated proteins from liver cytosol of diabetic animals was predicted by their increased relative rates of degradation and their unchanged relative rates of synthesis. It should be noted that the depletion of small basic non-glycosylated proteins in diabetes is a general trend only, just as our previous studies showed the enhanced degradation of these classes of proteins is only a general trend (Dice *et al.*, 1978). For example, despite the highly significant tendency to deplete small or basic proteins in diabetes, the smallest and most basic polypeptides appeared to be exceptions to this generalization (Figs. 2 and 4). Furthermore, the depletion of certain protein fractions in diabetes may also reflect decreased synthesis in this condition (e.g. proteins of mol.wt. 24 000; Fig. 1a).

Whether changes in protein composition are related to the severity or duration of diabetes requires further examination. Our preliminary results suggest that depletion of small basic non-glycosylated proteins may occur only during net protein catabolism, since one diabetic animal that did not lose weight after insulin withdrawal (rat no. 1, Table 1) showed no changes in protein composition. The duration of diabetes may be an especially important factor in our studies since catabolism of liver proteins is enhanced during the first 2 days after insulin withdrawal (Garlick, 1980) but then falls below normal by 4 days. Most of our experiments were restricted to animals with their insulin withdrawn for 3 days since this was the interval used for our previous studies of protein catabolism (Dice *et al.*, 1978). However, preliminary experiments indicate similar changes in protein composition are also evident after 2 or 4 days of insulin withdrawal.

Factors in addition to selectively enhanced degradation of small basic non-glycosylated pro-

teins may theoretically contribute to their loss from liver cytosol during diabetes. These classes of proteins could be modified post-translationally in diabetes so that they become larger, more acidic and/or carbohydrate-containing. For example, non-enzymic glycosylation of proteins from erythrocytes (Bunn *et al.*, 1978; Miller *et al.*, 1980) or serum (Kennedy *et al.*, 1979; Day *et al.*, 1980) occurs in proportion to the diabetic hyperglycaemia. However, whether the intracellular glucose concentration rises in diabetic liver is unclear, and the slow time course of glycosylation probably could not account for the observed change in the proportion of non-glycosylated proteins in liver cells from diabetic animals (Bunn *et al.*, 1978). Furthermore, no evidence exists to our knowledge for preferential modification of small proteins or basic proteins in diabetes. Finally, several studies using cells in culture have confirmed that long-lived proteins are selectively degraded in response to insulin deprivation (Goldberg & St. John, 1976; Dice & Goldberg, 1981; L. Bourret & J. F. Dice, unpublished work), so we conclude that such preferential catabolism is likely to be a major contributor to the observed changes in protein composition during diabetes. It is interesting that the preferentially enhanced degradation of non-glycosylated proteins has been generally overlooked as a factor that may contribute to the observed accumulation of glycoproteins during diabetes.

Selective degradation and depletion of small basic non-glycosylated proteins may be physiologically advantageous in conditions such as diabetes for several reasons. The enhanced proteolysis provides amino acids to be used directly as energy sources or to be converted into glucose through gluconeogenesis (Felig, 1975; Goldberg & Chang, 1978). By selectively degrading long-lived proteins (small basic and/or non-glycosylated proteins) in diabetes, the organism derives the needed amino acids without sacrificing rapidly-turning-over enzymes that would be important for continued metabolic adaptability. Several investigators have shown that enzymes important in metabolic regulation tend to have short half-lives, whereas those that are relatively unimportant in metabolic control are long-lived (Segal & Kim, 1965; Berlin & Schimke, 1965; Goldberg & St. John, 1976).

The selective degradation of small basic non-glycosylated proteins may also be responsible for decreased concentrations of certain specific proteins in liver of diabetic animals. For example, the concentration of glyceraldehyde 3-phosphate dehydrogenase, a glycolytic enzyme, is decreased in diabetes (Manchester, 1970), and this enzyme is rather small (34 000-mol.wt. subunits; Harris & Perham, 1965) and basic (pI = 8.1; Smith & Velick, 1972). If the selective degradation of certain protein

classes applies to organelles as well as to cytosolic proteins, the degradation of small basic non-glycosylated proteins may also contribute to reports of decreased cytochrome concentrations in mitochondria from diabetic rats (Matsubara *et al.*, 1971). We wish to stress once again, however, that the tendency to accelerate degradation of small basic non-glycosylated proteins in diabetes is a general trend only. For example, phosphofructokinase is degraded especially rapidly in diabetic animals (Dunaway *et al.*, 1978) and this enzyme is large (80000-mol.wt. subunits; Dunaway *et al.*, 1978) and acidic (pI = 5.0; Malamud & Drysdale, 1978). In these cases, the dissociation of a polypeptide stabilizing factor (Dunaway *et al.*, 1978) or of substrates or coenzymes (Goldberg & Dice, 1974) may be the primary determinant of the enzyme's more rapid degradation in diabetes.

Our initial experiments indicate that basic serum proteins are also selectively degraded in diabetes (Dice *et al.*, 1978), and basic serum proteins are depleted in this disease (J. F. Dice, unpublished work). These findings may be clinically useful in that measurements of the proportion of acidic and basic proteins in serum may provide an indication of the degree to which protein catabolism is enhanced in diabetic patients. Such information might be useful in helping diabetics maintain proper protein balance and in evaluating the degree of diabetic control achieved during the preceding few days.

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