Polyamine and amino acid content, and activity of polyamine-synthesizing decarboxylases, in liver of streptozotocin-induced diabetic and insulin-treated diabetic rats

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1. Concentrations of polyamines, amino acids, glycogen, nucleic acids and protein, and activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, were measured in livers from control, streptozotocin-diabetic and insulin-treated diabetic rats. 2. Total DNA per liver and protein per mg of DNA were unaffected by diabetes, whereas RNA per mg of DNA and glycogen per g of liver were decreased. Insulin treatment of diabetic rats induced both hypertrophy and hyperplasia, as indicated by an increase in all four of these constituents to or above control values. 3. Spermidine content was increased in the livers of diabetic rats, despite the decrease in RNA, but it was further increased by insulin treatment. Spermine content was decreased by diabetes, but was unchanged by insulin treatment. Thus the ratio spermidine/spermine in the adult diabetic rat was more typical of that seen in younger rats, whereas insulin treatment resulted in a ratio similar to that seen in rapidly growing tissues. 4. Ornithine decarboxylase activity was variable in the diabetic rat, showing a positive correlation with endogenous ornithine concentrations. This correlation was not seen in control or insulin-treated rats. Insulin caused a significant increase in ornithine decarboxylase activity relative to control or diabetic rats. 5. *S*-Adenosylmethionine decarboxylase activity was increased approx. 2-fold by diabetes and was not further affected by insulin. 6. Hepatic concentrations of the glucogenic amino acids, alanine, glutamine and glycine were decreased by diabetes. Their concentrations and that of glutamate were increased by injection of insulin. Concentrations of ornithine, proline, leucine, isoleucine and valine were increased in livers of diabetic rats and were decreased by insulin. Diabetes caused a decrease in hepatic concentration of serine, threonine, lysine and histidine. Insulin had no effect on serine, lysine and histidine, but caused a further fall in the concentration of threonine.

A eukaryotic cell that has been stimulated to grow by dietary or hormonal means initiates a co-ordinate set of metabolically related events. This regulatory programme is referred to as the positive pleiotypic response (Hershko *et al.*, 1971). The original description included increased RNA synthesis and an increase in polyribosome aggregation with an increased capacity for protein biosynthesis and a decrease in protein degradation. There is also an increase in the uptake and phosphorylation of certain nucleic acid precursors, as well as in the transport of glucose. It has been suggested that an increase in the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17), giving rise to increased spermidine synthesis, is also part of the pleiotypic response (Hogan *et al.*, 1974; Russell *et al.*, 1976). Although insulin is considered to be the

classical positive pleiotypic effector in many tissues (Hershko *et al.*, 1971), relatively little information is available on the influence of insulin on polyamine synthesis or concentration. Panko & Kenney (1971) observed an increase in hepatic ornithine decarboxylase activity 4 h after injection of insulin into adrenalectomized rats. Richman *et al.* (1971) performed a similar experiment in two hypophysectomized rats and observed a slight, but not significant, increase. Mallette & Exton (1973) observed an increase in ornithine decarboxylase activity when livers were perfused with insulin, and Hogan *et al.* (1974) observed that addition of insulin to the culture medium could increase ornithine decarboxylase activity in hepatoma cells. During the course of the present investigation, Sochor *et al.* (1978) reported that rats in which diabetes had been

induced by injections of alloxan had low activity of hepatic ornithine decarboxylase. Levine *et al.* (1978) reported that diabetes induced by streptozotocin caused increased hepatic ornithine decarboxylase activity, and the activity could be returned to normal by insulin injections. None of these investigators measured polyamine concentrations or other enzymes of polyamine synthesis.

Diabetes has been shown to result in altered amino acid concentrations in rat liver (Kirsten *et al.*, 1961; Williamson *et al.*, 1967; Bloxam, 1972). The effect of insulin replacement, however, was not determined in any of these earlier studies. It has been suggested that the concentration of several amino acids can influence ornithine decarboxylase activity in hepatoma cells (Hogan & Murden, 1974). Thus any alterations of hepatic ornithine decarboxylase activity observed during insulin deficiency or replacement could be due to insulin itself or could be an indirect result of diabetes-induced or insulin-induced changes in the concentrations of certain amino acids.

The aim of the present investigation was to study the role of insulin in the control of polyamine concentrations in rat liver *in vivo*, and to relate changes in polyamine concentrations to other observed effects of insulin, including changes in concentration of nucleic acids, glycogen and amino acids. This paper reports that insulin replacement induces hypertrophy and hyperplasia in liver of diabetic rats and causes a concomitant increase in the activity of ornithine decarboxylase and in the spermidine content of liver. Increased activity of *S*-adenosylmethionine decarboxylase (*S*-adenosylmethionine carboxy-lyase, EC 4.1.1.50) and decreased content of spermine were observed in liver of diabetic rats, but insulin did not reverse these changes.

Materials and methods

Animals

Adult male rats of the Sprague-Dawley strain were purchased from Canadian Breeding Laboratories, St. Constant, Quebec, Canada. All rats had free access to Purina rat chow (Purina Ralston of Canada, Don Mills, Ontario, Canada) and water. Rats were starved overnight and diabetes was induced by a single subcutaneous injection of streptozotocin (75 mg/kg body wt.), dissolved in 0.15 M-NaCl/0.01 M-sodium citrate (pH 4.5) immediately before use. Control rats received an injection of buffer alone. Urinary glucose and ketones were assessed by using Clinistix tapes and Acetest tablets (Ames Co., Miles Laboratories, Rexdale, Ontario, Canada) respectively. All diabetic rats showed negligible weight gain in the absence of insulin replacement. Blood glucose was consistently

in excess of 22 mM. Slight focal necrosis in liver has been reported 3–4 days after injection of streptozotocin (Junod *et al.*, 1967), and thus animals were left at least 2 weeks after injection of streptozotocin to allow them to recover from any liver damage induced by the drug. Rats were killed 2–6 weeks after induction of diabetes. Insulin-treated animals received either two subcutaneous injections per day (09:00h, 18:00h) of crystalline zinc insulin suspension (100 µg/100 g body wt.) or a single subcutaneous injection per day of 4 units of protamine zinc insulin. The other animals received injections of 0.15 M-NaCl only. Rats were killed between 11:00 and 13:00h of the fourth day of injections. This was 2h after the final injection of crystalline insulin or vehicle, or 18h after the final injection of protamine zinc insulin or vehicle. There were no qualitative differences in any variable between these two insulin regimens, and thus the results were pooled.

Chemicals

Zinc insulin crystals (bovine, lot no. 1125; 24.7 units/mg) were a gift from Connaught Laboratories, Willowdale, Ontario, Canada. Protamine zinc insulin (40 units/ml) was purchased from Connaught Laboratories. Streptozotocin was a gift of Dr. William Dulin, Upjohn Research Laboratories, Kalamazoo, MI, U.S.A. Calf thymus DNA (type V) and *Torula* yeast RNA (type IV) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Omnifluor, L-[1-¹⁴C]ornithine (46 mCi/mmol) and *S*-adenosyl-L-[carboxy-¹⁴C]methionine (50.7 mCi/mmol) were from New England Nuclear, Dorval, Quebec, Canada. Diphenylamine and acetic acid (both AnalaR grade) were from BDH Chemicals, Poole, Dorset, U.K., and orcinol monohydrate was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other chemicals were of the highest grade available and were from Sigma. Centre wells and stoppers used in ¹⁴CO₂ trapping were from Kontes Glass Co., Vineland, NJ, U.S.A.

Preparation of liver extracts

Rapidly frozen tissue. Rats were killed by cervical dislocation. The liver was rapidly removed and frozen between aluminium clamps precooled in liquid nitrogen (Wollenberger *et al.*, 1960). The average time between dislocation of the neck and deep-freezing of the tissue was 15 s. Frozen liver was pulverized in a mortar to a fine powder with frequent additions of liquid nitrogen. One portion of the powder was weighed in a precooled centrifuge tube and ice-cold 30% (w/v) KOH (10 ml/g of liver) was added. Samples were immediately homogenized in the centrifuge tube, with a motor-driven Teflon pestle, and subsequently used for glycogen assay. A second portion of the powdered liver was weighed in a precooled centrifuge tube and ice-cold 12% (w/v)

HClO₄ (4 ml/g of liver) was added. Samples were immediately homogenized and precipitated protein and nucleic acid removed by centrifugation at 2000 g for 20 min at 4°C. The supernatant was adjusted to pH 2.0–2.2 by addition of NaOH before use for amino acid and polyamine analysis.

Homogenized tissue. The remaining liver was weighed and placed in 10 ml of ice-cold homogenizing medium {0.25 M-sucrose/2 mM-EDTA/5 mM-dithiothreitol/2 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4} per g of tissue. The liver was chopped with scissors and homogenized in a smooth-glass Potter–Elvehjem homogenizer at approx. 500 rev./min by five to six strokes of a motor-driven loose-fitting Teflon pestle (clearance 0.13–0.18 mm). A portion of homogenate was centrifuged at 105 000 g for 70 min to remove organelles. The supernatant of this fraction is referred to as cytosol.

Analysis of polyamines and amino acids

For amino acid analysis, the HClO₄ extract of rapidly frozen liver was applied to a Beckman amino acid analyser (Beckman Bulletin 121M-TB-013, 1976). For polyamine analysis, HClO₄ extracts were first diluted with an equal volume of sodium citrate buffer (0.067 M, pH 2.2) and then applied to a Beckman amino acid analyser, as described previously (Hall *et al.*, 1978).

Assay of enzymes

Ornithine decarboxylase activity in cytosol was measured by following release of ¹⁴CO₂ from L-[1-¹⁴C]ornithine, as described previously (Murphy & Brosnan, 1976). Reaction mixtures contained 0.1 μmol of pyridoxal phosphate, approx. 5 mg of liver protein, 50 μmol of Tris (pH 7.3), 0.2 μmol of EDTA and 0.4 μmol of L-ornithine (1 μCi/μmol), in a final volume of 2.0 ml.

Putrescine-dependent *S*-adenosylmethionine decarboxylase in cytosol was assayed by following the release of ¹⁴CO₂ from *S*-adenosyl-L-[carboxy-¹⁴C]-methionine (Symonds & Brosnan, 1977). *S*-Adenosyl-L-methionine was present in the incubation medium at a concentration of 0.2 mM and a specific radioactivity of 0.5 μCi/μmol. Assays were carried out at 37°C for 15 min, since significant disappearance of *S*-adenosyl-L-methionine was observed in some extracts with longer incubation times. This resulted in a loss of linearity of the enzyme assay with time, owing to the fall in substrate concentration. Putrescine concentration was saturating (2.5 mM).

Analysis of blood glucose and liver glycogen

Blood samples were obtained from the tail 24 h before death, and placed in heparinized tubes. They were immediately deproteinized by addition of

Ba(OH)₂ and ZnSO₄, followed by centrifugation (2000 g, 20 min; 4°C) of the resulting precipitate, and the supernatant was then assayed for glucose by the glucose oxidase method (Glucose No. 510; Sigma).

Extraction and hydrolysis of glycogen were carried out essentially as described by Hassid & Abraham (1957). KOH extracts of rapidly frozen tissues were heated at 98–100°C for 2 h, glycogen was precipitated by addition of 1.2 vol. of ethanol (95%, v/v) and the precipitated glycogen was hydrolysed in H₂SO₄ (2 M) for 90 min at 98–100°C. The hydrolysate was neutralized with NaOH and glucose was measured by the glucose oxidase method.

Analysis of nucleic acids and protein

Liver homogenate was used for these determinations. DNA was extracted into hot HClO₄, as described by Schneider (1945), and the concentration was determined with diphenylamine reagent (Burton, 1956) with calf thymus DNA as standard. RNA was precipitated with 0.5 M-HClO₄ and hydrolysed in 0.3 M-KOH, as described by Seiler & Schmidt-Glenewinkel (1975). The orcinol method (Ashwell, 1957) was used to determine RNA, with *Torula* yeast RNA as standard. Protein (in homogenates and cytosol) was determined as described by Lowry *et al.* (1951), after solubilization of membranous material with deoxycholate (Jacobs *et al.*, 1956). Bovine serum albumin was used as standard. It was essential to include buffer containing dithiothreitol in the standards and blanks, since it gives colour in the Lowry reaction.

Results and discussion

Streptozotocin-injected rats had blood glucose concentrations in excess of 22 mM, with an average concentration of 28.5 mM. All diabetic rats showed glucosuria (4+ by Clinistix), but were negative for ketones in the urine. After injection of insulin, all diabetic rats showed a decrease in blood glucose concentration and in degree of glucosuria (trace to 2+). During the first week after injection of streptozotocin, all diabetic rats lost weight (approx. 6 g/day); thereafter the weight loss slowed to approx. 1 g/day for the duration of the experiment. At the time of death, the body weight of the diabetic animals averaged 62% of that of their age-matched controls. Diabetic rats treated with insulin gained an average of 6 g/day. All of these animals showed positive weight gain over the 3 days of hormone treatment.

Liver growth

The liver of the diabetic animals was approx. 15% smaller than that of their age-matched controls, but

Table 1. *Glycogen, DNA, RNA, protein and polyamine contents of liver from control, streptozotocin-induced and insulin-treated diabetic rats*

Values are the means $\pm$ S.D. for n rats. Details of analysis are given in the Materials and methods section. * $P < 0.05$ compared with control; † $P < 0.05$ compared with diabetic.

	Control ($n = 11$)	Diabetic ($n = 17$)	Insulin-treated diabetic ($n = 8$)
Glycogen (mg/g of liver)‡	53.2 $\pm$ 3.5	10.4 $\pm$ 3.0*	87.7 $\pm$ 31.3*†
DNA (mg/g of liver)	1.32 $\pm$ 0.10	1.44 $\pm$ 0.11*	0.94 $\pm$ 0.12*†
RNA (mg/mg of DNA)	4.74 $\pm$ 0.43	3.77 $\pm$ 0.40*	5.07 $\pm$ 0.34†
Protein (mg/mg of DNA)	150 $\pm$ 16	152 $\pm$ 13	181 $\pm$ 15*†
Spermidine (nmol/mg of DNA)	491 $\pm$ 45	602 $\pm$ 92*	872 $\pm$ 119*†
Spermine (nmol/mg of DNA)	608 $\pm$ 77	499 $\pm$ 70*	478 $\pm$ 74*
Spermidine/spermine	0.82 $\pm$ 0.08	1.22 $\pm$ 0.20*	1.87 $\pm$ 0.39*†

‡ $n = 4$ for each group of rats for glycogen only.

the liver weight as a proportion of body weight (hepatosomatic index) was significantly increased over controls (control 3.4 ± 0.1 ; diabetic 4.1 ± 0.5 ; means $\pm$ S.D. for four rats). This is due to the loss of muscle and adipose-tissue mass without a concomitant decrease in liver weight. The total DNA content of liver from diabetic rats was not significantly less than that of controls. Insulin treatment resulted in a marked increase in liver size, to approximately double the pre-injection weight. The hepatosomatic index was also increased to 7.0 ± 1.0 (four rats), indicating that the increase in liver weight far exceeded the increased weight gain of the animal as a whole. There was growth (or hyperplasia) of the liver, as indicated by a 27% increase in total liver DNA. Similar increases in liver weight and DNA content were also reported by Steiner & Williams (1959) for alloxan-diabetic rats. Steiner and co-workers (Younger *et al.*, 1966) subsequently showed that the increase in DNA was accompanied by cell division, and that the hypertrophic response was comparable in magnitude with that observed after partial hepatectomy. Weber *et al.* (1972) also observed that insulin treatment of diabetic rats did not change the DNA content per liver cell, but that the cellularity of the liver and the total DNA content were increased.

Glycogen content, given in Table 1, was very low in diabetic rats and was markedly increased after insulin treatment. This is in agreement with previous reports by Steiner & Williams (1959) and Khandelwal *et al.* (1977). Thus the dose of insulin used in the present investigation had a marked effect on hepatic carbohydrate metabolism. Although total lipid was not measured in this series of experiments, the work of Osborn *et al.* (1953) would indicate that it was likely that there was also increased total lipid in livers of insulin-treated diabetic rats. Since there were such marked changes in glycogen and lipid

content, and thus of liver weight, we have used DNA, as an index of cellularity, to serve as a reference against which other components could be expressed.

Concentration of nucleic acids, protein and polyamines

Table 1 gives the RNA and protein contents of livers from experimental animals, relative to DNA. There was a significant decrease in hepatic RNA content in diabetic rats. Pain *et al.* (1974) found no change in total RNA in liver of diabetic rats, but their data are expressed per g of liver and thus changes in other components could obscure changes in RNA. Total hepatic protein content was unchanged in our diabetic rats. It has been reported by Peavy *et al.* (1978) that liver protein metabolism *in vivo* is less affected by insulin deficiency than is muscle, where synthesis of protein is decreased and degradation increased (Morgan *et al.*, 1972), and thus marked muscle wasting results.

Treatment of diabetic rats with insulin resulted in hepatic cell hypertrophy, as indicated by increased RNA and protein content, relative to DNA. Insulin has been shown to increase synthesis of both RNA (Steiner & King, 1966) and protein (Reaven *et al.*, 1973) in rat liver, and to decrease protein degradation (Mortimore & Mondon, 1970).

Hepatic spermidine content increased in diabetic rats, despite the decrease in RNA (Table 1). This is in contrast with the generally accepted hypothesis that changes in spermidine and RNA contents in tissues always move in concert (Russell *et al.*, 1976). Both of these changes appear to occur early in diabetes and to reach a new steady state, since there appears to be no further change from 2 to 6 weeks after streptozotocin injection.

Insulin treatment results in a comparable increase in RNA and spermidine (40 and 45% respectively),

as has previously been shown in other rapidly growing tissues (Raina *et al.*, 1966; Caldarera & Moruzzi, 1970; Brosnan *et al.*, 1978).

There was a marked fall in the spermine content in livers of diabetic rats, comparable with the rise in spermidine. In previous studies, spermine has been found to be decreased or unchanged during rapid growth (Russell & Durie, 1978) and to increase as growth slowed (Brosnan *et al.*, 1978). Nucleic acid and protein data given in Table 1 would indicate that the liver of the diabetic rat is not growing more rapidly than that of intact animals, although the spermine content has fallen significantly and spermidine is increased. The liver of the diabetic rat appears to have returned to a pattern of polyamine concentrations typical of younger animals, with spermidine exceeding spermine (Brosnan *et al.*, 1978). It is possible that these changes are not due to insulin deficiency itself, since insulin treatment does not affect the spermine concentration and it causes a further increase in spermidine. Thus the elevated spermidine/spermine ratio seen in liver of diabetic rats is further increased to that characteristic of very rapidly growing tissues (Russell & Durie, 1978). Suckling and diabetic rats both have high plasma glucagon/insulin ratios (Beaudry *et al.*, 1977; Gerich *et al.*, 1976), with high hepatic spermidine/spermine ratios. It is possible that the depressed spermine content is due to elevated glucagon rather than to depressed insulin concentrations.

Concentration of amino acids

Table 2 gives the amino acid concentrations in

livers of control, diabetic and insulin-treated diabetic rats. Aspartate is not accurately measured by this method, since it is co-eluted with glutathione. Asparagine and tryptophan are at the limits of detection and thus cannot be accurately quantified.

The principal gluconeogenic amino acids, alanine and glutamine, were decreased in concentration in diabetic animals, as previously shown by Williamson *et al.* (1967). They also observed a decrease in glutamate concentration, whereas Bloxam (1972) observed an increase and Kirsten *et al.* (1961) had wide fluctuations in their glutamate results. Our glutamate results in control and diabetic rats also show wide scatter, but do not differ statistically from one another. Insulin returned the concentration of these gluconeogenic amino acids to normal, and elevated the glutamate concentration.

Serine and threonine concentrations were also decreased in the livers of diabetic animals. Similar findings had been obtained previously by Kirsten *et al.* (1961), Williamson *et al.* (1967) and Bloxam (1972). Serine dehydratase (Freedland & Avery, 1964) and serine-pyruvate aminotransferase (Rowell *et al.*, 1973) have been shown to be increased in activity by diabetes, and thus there could be an increased degradation of these amino acids in liver of diabetic animals. The present study, however, casts doubt on the role of insulin in this process. Treatment of diabetic animals with insulin had no significant effect on serine concentration and caused a further significant fall in threonine concentration. Glucagon has been shown to induce serine dehydratase (Jost *et al.*, 1968) and serine-pyruvate

Table 2. Amino acid contents of liver from control, streptozotocin-induced diabetic and insulin-treated diabetic rats. Values are the means $\pm$ S.D. Details of analysis are given in the Materials and methods section. All results are expressed as μ mol of amino acid/g of liver. * $P < 0.05$ compared with control; † $P < 0.05$ compared with diabetic.

Amino acid	Control (n = 11)	Diabetic (n = 17)	Insulin-treated diabetic (n = 8)
Threonine	0.36 $\pm$ 0.18	0.18 $\pm$ 0.04*	0.12 $\pm$ 0.07*†
Serine	0.65 $\pm$ 0.24	0.23 $\pm$ 0.07*	0.30 $\pm$ 0.23*
Glutamine	5.29 $\pm$ 0.76	3.66 $\pm$ 0.60*	5.44 $\pm$ 1.16†
Proline	0.09 $\pm$ 0.05	0.15 $\pm$ 0.06*	0.08 $\pm$ 0.04†
Glutamate	2.85 $\pm$ 0.70	2.55 $\pm$ 0.78	4.44 $\pm$ 0.82*†
Glycine	2.35 $\pm$ 0.42	1.29 $\pm$ 0.30*	1.91 $\pm$ 0.41*†
Alanine	2.29 $\pm$ 1.12	0.74 $\pm$ 0.32*	2.56 $\pm$ 0.53†
Valine	0.19 $\pm$ 0.05	0.81 $\pm$ 0.29*	0.22 $\pm$ 0.08†
Methionine	0.08 $\pm$ 0.03	0.09 $\pm$ 0.03	0.11 $\pm$ 0.03
Isoleucine	0.15 $\pm$ 0.04	0.36 $\pm$ 0.10*	0.14 $\pm$ 0.07†
Leucine	0.24 $\pm$ 0.05	0.64 $\pm$ 0.19*	0.23 $\pm$ 0.08†
Tyrosine	0.09 $\pm$ 0.02	0.07 $\pm$ 0.02	0.06 $\pm$ 0.02*
Phenylalanine	0.08 $\pm$ 0.02	0.07 $\pm$ 0.01	0.07 $\pm$ 0.02
Ornithine	0.36 $\pm$ 0.10	0.49 $\pm$ 0.18*	0.25 $\pm$ 0.12*†
Lysine	0.54 $\pm$ 0.17	0.37 $\pm$ 0.14*	0.36 $\pm$ 0.14*
Histidine	0.70 $\pm$ 0.07	0.50 $\pm$ 0.08*	0.55 $\pm$ 0.09*
Arginine	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01

aminotransferase (Rowell *et al.*, 1973; Fukushima *et al.*, 1978) in rat liver, and thus the increased activity of these enzymes in diabetic rats could be due to the elevated glucagon concentration seen in these animals.

We also observed a similar decrease in concentration of lysine and of histidine in liver of diabetic rats and a failure of insulin to return the concentrations to normal. It has been reported that histidine-pyruvate aminotransferase is identical with serine-pyruvate aminotransferase (Noguchi *et al.*, 1976). Thus increased activity of degradative enzymes could also explain the observed decrease in concentration of these amino acids in diabetic rats.

There was a marked increase in the concentration of the branched-chain amino acids, leucine, isoleucine and valine, in liver of diabetic rats. This is in agreement with the results of Bloxam (1972), who used streptozotocin-diabetic rats, but in contrast with those of Kirsten *et al.* (1961), who studied alloxan-induced diabetes in rats. These essential amino acids could be derived both from increased proteolysis in various tissues in diabetic animals (Berger *et al.*, 1978) and from the diet since one of the characteristics of streptozotocin-induced diabetes is hyperphagia (Booth, 1972). Elevated concentrations of unesterified fatty acids or ketones in perfused rat heart have been reported by Buffington *et al.* (1979) to inhibit branched-chain oxo acid dehydrogenase, an enzyme required for the metabolism of branched-chain amino acids. Both fatty acids and ketones are increased in diabetic animals, and thus a decreased oxidation of branched-chain amino acids could also occur. As a result of increased production and decreased utilization of these amino acids, an increase in their concentration in the blood of diabetics would be predicted, and, in fact, appears to be a consistent finding in rats (Bloxam, 1972) or humans (Berger *et al.*, 1978). Liver *in vivo* or *in vitro* (perfused) has been shown to maintain equal concentrations of each branched-chain amino acid in plasma and in liver (Bloxam, 1972; Schimassek & Gerok, 1965). Thus an increased concentration of these amino acids in plasma of diabetics would be expected to lead to an increased concentration in liver. When diabetic rats were treated with insulin, the concentrations of all three of these amino acids in liver were returned to normal, thus implicating insulin deficiency as the causative factor. In well-controlled diabetic humans, the concentration of branched-chain amino acids in plasma is in the normal range (Berger *et al.*, 1978).

Arginine and methionine are thought to be the ultimate amino acid precursors of the polyamines. No change was observed in the hepatic concentration of either of these amino acids in diabetic rats with or without insulin.

Enzymes of polyamine synthesis

Activities of ornithine decarboxylase and putrescine-dependent *S*-adenosylmethionine decarboxylase are given in Table 3. There was a large variation in ornithine decarboxylase activity in liver of diabetic rats, with the mean activity not significantly different from control. While the present study was in progress, Sochor *et al.* (1978) reported a decreased ornithine decarboxylase activity in liver of alloxan-diabetic rats maintained without insulin therapy for 2–3 weeks. Levine *et al.* (1978) observed an increased activity of hepatic ornithine decarboxylase in rats that had been rendered diabetic by injection of streptozotocin 4–8 days before death. We did not observe any correlation between duration of diabetes from 2 to 6 weeks and ornithine decarboxylase activity, but we did not look at shorter or longer times. We did observe a new higher steady-state concentration of spermidine, and an elevated activity of ornithine decarboxylase *in vivo* could be in agreement with this.

There was wide variability from one diabetic rat to the next both in the concentration of ornithine in liver and in the activity of ornithine decarboxylase. These data are plotted in Fig. 1. There is a positive correlation ($r = +0.86$) between ornithine concentration and ornithine decarboxylase activity in livers from diabetic rats, although this relationship was not evident in control or insulin-treated rats. Ornithine decarboxylase appears to have the shortest half-life of any liver enzyme studied to date (Goldberg & St. John, 1976), although little is known of the mechanism of its degradation. It has been suggested that increases in substrate or cofactor concentration can protect an enzyme from degradation (Goldberg & St. John, 1976). It appears possible that the elevated

Table 3. Ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activity of liver from control, streptozotocin-induced diabetic and insulin-treated diabetic rats

Values are the means $\pm$ S.D. for the numbers of rats shown in parentheses. Enzyme assays are described in the Materials and methods section. Activity of ornithine decarboxylase is given as pmol/h per mg of cytosol protein and that of putrescine-dependent *S*-adenosylmethionine decarboxylase as pmol/15 min per mg of cytosol protein. * $P < 0.05$ compared with control; † $P < 0.05$ compared with diabetic.

Animals	Ornithine decarboxylase	<i>S</i> -Adenosylmethionine decarboxylase
Control	61 $\pm$ 53 (11)	86 $\pm$ 19 (10)
Diabetic	154 $\pm$ 156 (17)	194 $\pm$ 79 (12)*
Insulin-treated diabetic	465 $\pm$ 391 (8)*†	157 $\pm$ 57 (8)*

Fig. 1. Ornithine decarboxylase activity measured *in vitro* as a function of liver content of ornithine. Each point represents data from a single liver. ○, Liver from control rats; ●, liver from diabetic rats; △, liver from insulin-treated diabetic rats. For details see the Materials and methods section.

ornithine concentration observed in liver of some diabetic rats could provide protection from degradation for ornithine decarboxylase and thus result in a fortuitous increase in enzyme activity in these rats. Bloxam (1972) did observe an increase in hepatic ornithine concentration in rats with the same duration of streptozotocin-induced diabetes as those in which Levine *et al.* (1978) reported an increased ornithine decarboxylase activity.

Increased concentrations of the branched-chain amino acids have been shown by Matsuzawa (1974) to inhibit ornithine-2-oxoglutarate aminotransferase, the first step in the catabolism of ornithine. Thus it is possible that the increased concentrations of leucine, isoleucine and valine in liver of diabetic rats inhibit metabolism of ornithine and thus result in an increased concentration of ornithine in liver. Injection of insulin resulted in a significantly increased ornithine decarboxylase activity. This is in agreement with previous observations that insulin is able to increase hepatic ornithine decarboxylase activity when injected *in vivo* (Panko & Kenney, 1971), perfused through liver (Mallette & Exton, 1973) or added to culture medium (Hogan *et al.*, 1974). It is not evident at present why there is a discrepancy

Fig. 2. Putrescine concentration as a function of ratio of activity of ornithine decarboxylase to *S*-adenosylmethionine decarboxylase in rat liver

Each point represents the mean of duplicate determinations in a single liver. The ratio of enzyme activities was calculated from data in Table 3. ○, Liver from control rats; ●, liver from diabetic rats; △, liver from insulin-treated diabetic rats. Correlation coefficient was +0.89. For details see the Materials and methods section.

between these results and those of Levine *et al.* (1978), who observed that insulin injected into diabetic rats lowered the elevated ornithine decarboxylase activity. It is noteworthy that we observe an increase in ornithine decarboxylase activity in the face of decreased substrate concentration (see Fig. 1).

The concentrations of several amino acids have been shown to influence activity of ornithine decarboxylase in liver cells. Fausto (1971) found that giving rats histidine or arginine could increase ornithine decarboxylase activity in liver, but he thought that the response was hormonally mediated and thus was not due to a direct effect of the amino acid on the liver. Hogan & Murden (1974) were able to increase ornithine decarboxylase activity in hepatoma cells by adding amino acids, specifically glutamine, asparagine, serine, glycine and proline, to the culture medium. The concentration of these amino acids in the hepatoma cells was not determined, so it is not clear whether this effect was due to increased concentration of these amino acids inside the cells or in the medium. In liver

of diabetic animals (Table 2), glutamine, serine and glycine were significantly decreased, asparagine was still below the limits of detection and proline was increased. Insulin returned the glutamine, glycine and proline concentrations to normal, but increased the ornithine decarboxylase activity above that seen in either control or diabetic rats. Therefore the effect of insulin does not appear to be mediated by increased concentrations of these amino acids in the liver cell.

Putrescine-dependent *S*-adenosylmethionine decarboxylase activity was increased 2-fold by diabetes and remained unaffected by insulin treatment of diabetic animals. This was an unexpected observation, since we had previously shown that a single injection of insulin into starved rats caused an elevation in *S*-adenosylmethionine decarboxylase activity (Symonds & Brosnan, 1978). The increased ornithine decarboxylase activity measurable *in vitro* correlates with an increase in putrescine concentration *in vivo* ($r = +0.85$), especially when production of putrescine by ornithine decarboxylase exceeds the measurable activity of *S*-adenosylmethionine decarboxylase (Fig. 2). This could make the provision of decarboxylated *S*-adenosylmethionine rate-limiting for spermidine synthesis, and lead to accumulation of putrescine.

Conclusion

Insulin treatment of these diabetic rats caused a typical pleiotypic response in the liver. There was hypertrophy and hyperplasia and a concomitant increase in the activity of ornithine decarboxylase and in the tissue content of spermidine. However, it is clear that insulin lack was not the sole cause of the changes seen in diabetes, since insulin replacement reversed neither the decrease in hepatic spermine content nor the increase in hepatic activity of *S*-adenosylmethionine decarboxylase. It is therefore apparent that hormones other than insulin are important in determining the hepatic contents of the polyamines and the activities of the enzymes that synthesize them. In this connection, it is possible that glucagon plays a more important part than has been hitherto appreciated. Elevated glucagon concentrations may also account for the decreased hepatic concentrations of serine and threonine seen in livers of diabetic rats and for the inability of insulin treatment to reverse these changes.

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