Ornithine decarboxylase activity in insulin-deficient states

Cheryl A. CONOVER,*† S. Jaime ROZOVSKI,‡ Eva R. BELUR,† Thomas T. AOKI§ and Neil B. RUDERMAN†

†Division of Diabetes and Metabolism, University Hospital, The Evans Memorial Department of Medicine and Department of Physiology, Boston University Medical Center, 75 East Newton Street, Boston, MA 02118,

‡The Institute of Human Nutrition, Columbia University, New York, NY 10032, and §The Joslin Research Laboratory, Department of Medicine, Harvard Medical School, Boston, MA 02115, U.S.A.

(Received 30 May 1980/Accepted 14 July 1980)

The activity of ornithine decarboxylase, the rate-controlling enzyme in polyamine biosynthesis, was determined in tissues of normal control rats and rats made diabetic with streptozotocin. In untreated diabetic rats fed *ad libitum*, ornithine decarboxylase activity was markedly diminished in liver, skeletal muscle, heart and thymus. Ornithine decarboxylase was not diminished in a comparable group of diabetic rats maintained on insulin. Starvation for 48 h decreased ornithine decarboxylase activity to very low values in tissues of both normal and diabetic rats. In the normal group, refeeding caused a biphasic increase in liver ornithine decarboxylase; there was a 20-fold increase in activity at 3 h followed by a decrease in activity, and a second peak between 9 and 24 h. Increases in ornithine decarboxylase in skeletal muscle, heart and thymus were not evident until after 24–48 h of refeeding, and only a single increase occurred. The increase in liver ornithine decarboxylase in diabetic rats was greater than in normal rats after 3 h of refeeding, but there was no second peak. In peripheral tissues, the increase in ornithine decarboxylase with refeeding was diminished. Skeletal-muscle ornithine decarboxylase is induced more rapidly when meal-fed rats are refed after a period without food. Refeeding these rats after a 48 h period without food caused a 5-fold increase in ornithine decarboxylase in skeletal muscle at 3 h in control rats but failed to increase activity in diabetic rats. When insulin was administered alone or together with food to the diabetic rats, muscle ornithine decarboxylase increased to activities even higher than in the refed controls. In conclusion, these findings indicate that the regulation of ornithine decarboxylase in many tissues is grossly impaired in diabetes and starvation. They also suggest that polyamine formation *in vivo* is an integral component of the growth-promoting effect of insulin or some factor dependent on insulin.

The polyamines putrescine, spermidine and spermine are thought to be intricately involved in the regulation of nucleic acid and protein synthesis and cell growth (Jänne *et al.*, 1978). The initial step in their formation, the decarboxylation of ornithine to form putrescine, is catalysed by the enzyme ornithine decarboxylase. Ornithine decarboxylase is unique in that its half-life is 10–20 min, the shortest of any known mammalian enzyme. Furthermore, its activity generally parallels changes in putrescine and fluctuates rapidly and specifically in response to a variety of hormonal and metabolic factors that alter cell growth. Thus ornithine decarboxylase is markedly increased in tissues in which growth is

accelerated and its activity is usually low when the rate of growth is slow (Jänne *et al.*, 1978).

It is well known that diabetes and starvation diminish RNA and protein synthesis in most tissues and that there is impaired growth in these states (Wool *et al.*, 1968; Garlick *et al.*, 1975; Nakano, 1978; Li *et al.*, 1979). Despite this, relatively little is known about the effect of starvation on polyamine metabolism in tissues other than the liver (Fausto, 1969; Hayashi *et al.*, 1972; Domschke & Söling, 1973; Eloranta & Raina, 1977), and only two seemingly contradictory studies on the effects of diabetes have been reported (Levine *et al.*, 1978; Sochor *et al.*, 1978). Therefore we have attempted to assess whether the impaired growth characteristic of these states is associated with alterations in poly-

* To whom reprint requests should be addressed.

amine biosynthesis as reflected by ornithine decarboxylase activity. The present paper describes the response of ornithine decarboxylase to starvation and refeeding in various tissues of normal rats and the effect of diabetes on this response. In addition, we have investigated the possible regulatory role of insulin in the induction of ornithine decarboxylase in skeletal muscle after food ingestion.

Materials and methods

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) weighing 140–160 g were used in all studies. They were fed Purina laboratory chow and were kept in temperature-controlled animal quarters (23°C) with a 12h-light/12h-dark cycle (08:00h to 20:00h).

Materials

DL-[1-¹⁴C]Ornithine monohydrochloride (sp. radioactivity 52.8 Ci/mol) and Protosol were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Fluoropo (*o*-phthalaldehyde) was from Dionex Corporation (Sunnyvale, CA, U.S.A.) and ScintiVerse was from Fisher Scientific (Fairlawn, NJ, U.S.A.). Protamine/zinc/insulin (U-40) and crystalline pork insulin (Iletin U-100) were obtained from Eli Lilly Company (Indianapolis, IN, U.S.A.). Streptozotocin was kindly provided by Dr. W. E. Dulin, Upjohn Company, Kalamazoo, MI, U.S.A.

Experiments with treated and untreated diabetic rats

Diabetes was induced by an intraperitoneal injection of streptozotocin (80 mg/kg body wt.) administered after overnight starvation. After 36 h the average random blood glucose of these rats was 256 mg% compared with 90 mg% for control rats. Half of the diabetic rats were then maintained on 4 units of protamine/zinc/insulin per day administered subcutaneously at 15:00h for 6 days. The other diabetic rats and normal controls received an equal volume of saline. All rats were fed *ad libitum*.

Between 08:30h and 10:00h, and approx. 8 days after diabetes had been induced, rats were anaesthetized with pentobarbital (5 mg/100 g, intraperitoneally) and hindlimb tissue consisting mainly of gastrocnemius and posterior thigh muscle, the left lobe of the liver, the apex of the heart and the thymus were excised. The tissues were homogenized immediately in 3–4 ml of ice-cold 0.25 M-sucrose (pH 7.2) containing 1 mM-2-mercaptoethanol and 0.3 mM-disodium EDTA, with a Polytron homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.). This homogenate could be frozen for at

least 10 days without loss of ornithine decarboxylase activity. Pooled abdominal blood was collected for glucose measurements.

Starvation/refeeding experiments

Rats fed *ad libitum*. Diabetes was induced by an intraperitoneal injection of streptozotocin (80 mg/kg body wt.) as described above. Random serum glucose in untreated diabetic rats fed *ad libitum* averaged 521 mg% at 5 days after the injection, compared with 156 mg% in normal rats. After diabetes had been established for approx. 6 days, control and diabetic rats were starved for 48 h. They were then allowed free access to food and killed at various times thereafter. Blood was collected and tissues were excised and homogenized as described in the preceding section. In addition, the right and middle lobes of the liver and hindlimb muscle were frozen and saved for the determination of polyamines and nucleic acids.

Meal-fed rats. These experiments were similar to those carried out in rats fed *ad libitum* except that the rats used were meal-fed. Normal rats were allowed access to food from 09:00 to 12:00h for 4–5 days. At that time, diabetes was induced in half of the group by an intravenous injection of streptozotocin (60 mg/kg body wt.). Random blood glucose concentrations in the diabetic rats were 200–250 mg% compared with 70–90 mg% in the control animals. Control and diabetic rats were meal-fed for an additional 4–5 days. They were then starved for 48 h, after which they were re-fed for 3 h and/or injected intraperitoneally with crystalline insulin (0.2 unit/100 g body wt). Blood was collected and skeletal muscle was taken for analysis in rats killed at the end of the starvation period and after 3 h of refeeding.

Assay of ornithine decarboxylase

Tissue homogenates were centrifuged at 40000 g for 1 h at 3°C and the supernatant was used for determination of enzyme activity. Ornithine decarboxylase was assayed by measuring the evolution of ¹⁴CO₂ from [¹⁴C]carboxy-labelled ornithine by using a modification of the method described by Russell & Synder (1968). Incubations were carried out in Pyrex test tubes (100 mm × 16 mm) capped with rubber stoppers from which were suspended a polyethylene centre well (Kontes Glass Company, Vineland, NJ, U.S.A.). The centre wells contained 0.3 ml of Protosol as a CO₂-trapping reagent. The incubation mixture contained 50 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.2, 1 mM-EDTA, 50 μM-pyridoxal 5'-phosphate, 2.5 mM-dithiothreitol and 50 μM-L-ornithine (0.5 μCi of DL-[¹⁴C]ornithine), in a final volume of 1 ml. Tubes were incubated in a shaking water bath

at 37°C for 60 min, after which 0.5 ml of 40% trichloroacetic acid was injected to stop the reaction and to enhance conversion of bicarbonate into CO₂. They were then allowed to shake for an additional 30 min. The ¹⁴CO₂ that evolved was trapped in Protosol and counted in a Packard Tri-Carb liquid-scintillation spectrometer using ScintiVerse as the scintillation 'cocktail'. Supernatant protein was determined by the method of Lowry *et al.* (1951). Ornithine decarboxylase activity was found to be linear with enzyme concentration and assay time and is expressed as pmol of CO₂ released/mg of protein per 60 min.

Analyses

Putrescine and nucleic acids. Frozen liver and skeletal muscle were homogenized in 5 vol. of cold deionized water. Cold 0.6 M-HClO₄ (0.5 ml) was added to 1 ml of the homogenate and the mixture was centrifuged at 2000 g for 15 min. The supernatant was saved and the resultant pellet was extracted three times with 2 ml of 0.2 M-HClO₄. The combined supernatant fraction was saved for putrescine determinations. The pellet was extracted for DNA, which was determined as previously described (Rozovski *et al.*, 1978). Putrescine was measured by using a modification of the procedure described by Marton *et al.* (1974). Determinations were performed on a Beckman 120C amino-acid analyser equipped with a Dionex column (32 mm × 5 cm; Dionex Corporation, Palo Alto, CA, U.S.A.). Dionex DC-4A resin was used. A water-bath temperature of 60°C was maintained during the entire run. The polyamines were eluted from the column by using a citrate-based three-buffer system of increasing molarity. The column eluate was then treated with Fluoropo dissolved in 0.6 M-boric acid with the pH adjusted to 10.6. Fluorescence was

detected by using an Aminco fluorimeter (American Instruments, Silver Springs, MD, U.S.A.) and recorded on a dual-channel strip recorder. The minimum sensitivity of the methodology that gave reproducible results was 100 pmol; the average time of a run was 65 min.

Glucose. Blood and serum glucose were determined in a glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.).

Statistics

Analysis of variance and the Dunnett (1955) test were employed when comparing multiple groups with a single control. Differences between two groups were analysed by using the Student's *t* test for two independent samples. Results were considered statistically significant when *P* < 0.05.

Results

Effects of diabetes on ornithine decarboxylase activity

The effect of diabetes on ornithine decarboxylase activity in rats fed *ad libitum* is shown in Table 1. Diabetic rats were studied 8 days after having received streptozotocin and had a mean blood glucose concentration of 310 mg% at the time of death. They gained an average of 3 g/day, compared with 8 g/day for controls. In keeping with this decrease in weight gain, ornithine decarboxylase activity was diminished by 60% in liver and thymus and by 85% in heart and skeletal muscle of the diabetic rats. Treatment with insulin restored weight gain and ornithine decarboxylase activities to control values or greater. That the insulin-treated rats were sometimes hypoglycaemic is suggested by their low normal blood glucose concentration at the time of

Table 1. *Effect of diabetes on ornithine decarboxylase activity in rat tissues*

One group of diabetic rats received 4 units of protamine/zinc/insulin per day at 15:00–16:00 h for 6 days. Control and other diabetic rats received saline. All animals were fed *ad libitum*. Results are means ± S.E.M. See the Materials and methods section for details.

	Group n	Control 6	Diabetic 6	Diabetic + insulin 10
Weight gain (g/day)		8 ± 0.6	3 ± 1*	12 ± 0.3*
Blood glucose at time of death (mg%)		111 ± 4	310 ± 18*	56 ± 4*
Glycosuria (Diastix)		—	3–4+	—
Ornithine decarboxylase activity (pmol of CO ₂ released/h per mg of protein)				
Liver		13 ± 1.8	5 ± 0.4*	14 ± 2.3
Skeletal muscle		40 ± 6	6 ± 0.7*	48 ± 6
Heart		213 ± 22	32 ± 8*	298 ± 32
Thymus		37 ± 9	15 ± 3*	97 ± 17*

* Value significantly different from that of the control group (based on analysis of variance by the Dunnett procedure) at *P* < 0.05.

killing. As hypoglycaemia would cause hyperphagia, this might account for the increased weight gain and the very high thymic ornithine decarboxylase activity in this group.

Effect of starvation and refeeding on ornithine decarboxylase activity

Although the data in Table 1 demonstrate that ornithine decarboxylase activity is decreased in diabetic rats fed *ad libitum*, the interpretation of these findings is made difficult by the fact that measurements were carried out at a single point in time. There are two reasons for this. First, food ingestion markedly increases ornithine decarboxylase activity in many tissues (Hopkins *et al.*, 1972; Domschke & Söling, 1973; Maudsley *et al.*, 1976; McAnulty & Williams, 1977), and diabetes may alter the rats' pattern of food ingestion (Booth, 1972). Secondly, changes in ornithine de-

carboxylase activity may be transient (Jänne *et al.*, 1978). To eliminate these potential variables as much as possible, we measured changes in tissue ornithine decarboxylase in control and diabetic rats after starvation for 48 h and during the subsequent period of refeeding. The results are shown in Figs. 1 and 2. Values for ornithine decarboxylase in liver, heart and skeletal muscle before starvation were similar to those presented in Table 1 in both control and diabetic rats. In contrast, thymic ornithine decarboxylase activity was five times greater. This may have been due to the fact that the rats in the previous study had been injected with saline and handled daily. These stresses could have increased plasma glucocorticoids, which are known to decrease thymic ornithine decarboxylase activity (Beaven & DeJong, 1973). Starvation of control rats for 48 h caused a 70–90% decrease in ornithine decarboxylase in all of the tissues studied. Starvation also decreased ornithine decarboxylase in muscle, heart and thymus of the diabetic rats and differences in tissue ornithine decarboxylase between control and diabetic rats were no longer apparent.

Fig. 1. Effect of starvation and refeeding on liver ornithine decarboxylase activity in control and diabetic rats

Control (●) and diabetic (△) rats were starved for 48 h then refeed. Each point represents the mean $\pm$ S.E.M. for four to 16 rats. Where not shown the S.E.M. bar falls within the point. * indicates a significant difference between control and diabetic rats (based on Student's *t* test) at $P < 0.05$. See the Materials and methods section for details.

Fig. 2. Effect of starvation and refeeding on thymus, heart and skeletal-muscle ornithine decarboxylase activity in control and diabetic rats

Symbols: ●, control; △, diabetic. See the legend to Fig. 1 and the Materials and methods section for details.

Figs. 1 and 2 show the effect of refeeding on tissue ornithine decarboxylase. In normal rats, refeeding caused a biphasic increase in hepatic ornithine decarboxylase. There was a 20-fold increase in activity at 3 h, which was 7-fold above the value before starvation. This initial increase was followed by a decrease in activity, and then a second peak between 9 and 24 h (Fig. 1). In the experiment represented in Fig. 1, ornithine decarboxylase activity at 5 h was significantly lower than at 3 h. The difference between activities at 5 and 9 h was not statistically significant ($P < 0.2$), but in other experiments (results not shown) highly significant differences have been noted. In diabetic rats, the increase in liver ornithine decarboxylase was greater than in normal rats after 3 h of refeeding. However, there was no second peak between 9 and 24 h, although activity was still higher than at the end of the starvation period (Fig. 1). These changes in ornithine decarboxylase were closely associated with changes in its product, putrescine (Table 2).

In contrast, with liver, increases in ornithine decarboxylase were evident in muscle, heart and thymus only after 24–48 h of refeeding, and a single increase occurred (Fig. 2). In the diabetic rats, these increases were less marked. The diminished ornithine decarboxylase response in the diabetic group was not due to inadequate energy intake, since diabetic rats ate the same amount of food as control rats, as judged by similar increases in body weight over the first 9 h of refeeding. Interestingly, refeeding was associated with a decrease in the weight of the thymus in diabetic rats. The thymus of the diabetic rats when fed *ad libitum* weighed 41 ± 4 mg (mean $\pm$ S.E.M.) and was approximately the same 3 h after the onset of refeeding. Thereafter, however, the weight of the thymus decreased progressively so that at 96 h its mean weight was 5 ± 0 mg ($n = 4$). In contrast, the thymus of the control rats showed no change during refeeding and weighed 47 ± 3 mg ($n = 6$) at 96 h. These changes were not related to an increased severity of the diabetic state, since blood glucose concentrations at the time of death were (mean $\pm$ S.E.M.) 259 ± 9 , 346 ± 22 , 331 ± 16 and

332 ± 22 mg% in rats fed *ad libitum* and rats refed for 48, 72 and 96 h after starvation respectively. As reported by Levine & Hegarty (1977) and Nakano (1978) and confirmed in our studies (results not shown), refeeding either increased or caused relatively little change in the weight of liver, skeletal muscle and heart.

The response of ornithine decarboxylase in starved/refed rats was also studied after 72 and 96 h of refeeding (Table 3). These experiments were carried out on a different day and for this reason ornithine decarboxylase values in the *ad libitum* fed group and after 48 h of refeeding may be somewhat different from those presented in Figs. 1 and 2. The data reveal that ornithine decarboxylase attains its '*ad libitum*-fed' activity after different periods of refeeding in the four tissues studied. Thus, liver and muscle ornithine decarboxylase are increased after 48 h of refeeding and they diminish to values found in rats fed *ad libitum* by 72 h. Thymic ornithine decarboxylase is increased after 72 h of refeeding, then decreases to values for rats fed *ad libitum* by 96 h. In contrast, ornithine decarboxylase activity was not significantly different from basal values in heart by 48 h.

Effect on insulin on muscle ornithine decarboxylase

An obvious question is whether insulin can restore the food-induced increase in ornithine decarboxylase to normal in diabetic rats. In previous studies, we found that food ingestion causes muscle ornithine decarboxylase to increase markedly within 3 h in rats trained to eat their food in a single meal (Rozovski *et al.*, 1979). Thus, they are better suited to study the acute effects of insulin than are rats in which food-induced changes in ornithine decarboxylase are not seen for 24–48 h. As shown in Table 4, starvation caused skeletal-muscle ornithine decarboxylase to decrease in the meal-fed rats to the same low values seen in control and diabetic rats that had previously been fed *ad libitum* (Figs. 1 and 2). As reported previously, activity increased 5-fold in normal rats within 3 h of the onset of refeeding. Such an increase did not occur in the diabetic rats.

Table 2. Effect of starvation and refeeding on liver putrescine in control and diabetic rats

Putrescine was measured in the same control and diabetic rats for which ornithine decarboxylase values are presented in Fig. 1. Each value is the mean $\pm$ S.E.M. for the numbers of observations shown in parentheses.

Time refed (h)	Ornithine decarboxylase activity (pmol of CO ₂ released/mg of protein per h)		Putrescine (pmol/ μ g of DNA)	
	Control	Diabetic	Control	Diabetic
0	7 ± 0.9 (7)	5 ± 0.6 (6)	10 ± 2 (3)	9 ± 2 (3)
3	160 ± 16 (12)	269 ± 48 (9)	112 ± 30 (4)	172 ± 52 (4)
9	135 ± 20 (4)	46 ± 18 (8)	75 ± 15 (4)	30 ± 7 (3)

Table 3. *Effect of starvation and refeeding on tissue ornithine decarboxylase activity in control rats*
Control rats fed *ad libitum* were starved for 48 h, then refed. Results are means $\pm$ S.E.M. * indicates a value significantly different from that of the group fed *ad libitum* (based on analysis of variance by the Dunnett procedure) at $P < 0.05$.

	n	Ornithine decarboxylase activity (pmol of CO ₂ released/h per mg of protein)			
		Liver	Muscle	Heart	Thymus
Fed <i>ad libitum</i>	12	17 $\pm$ 3	26 $\pm$ 3	215 $\pm$ 24	187 $\pm$ 30
Refed for 48 h	5	35 $\pm$ 10*	81 $\pm$ 13*	150 $\pm$ 12	226 $\pm$ 26
Refed for 72 h	7	11 $\pm$ 0.4	48 $\pm$ 17	207 $\pm$ 27	350 $\pm$ 60*
Refed for 96 h	6	13 $\pm$ 2	56 $\pm$ 1	187 $\pm$ 2	229 $\pm$ 35

Table 4. *Effect of refeeding and insulin on skeletal-muscle ornithine decarboxylase activity in meal-fed control and diabetic rats*

All rats were meal-fed and starved for 48 h. One group of control rats was refed. Diabetic rats were refed or administered crystalline insulin (0.2 unit/100 g body wt.) either alone or 15 min after the onset of refeeding. Values are means $\pm$ S.E.M. * indicates a value significantly different from that of the starved group (based on analysis of variance by the Dunnett procedure) at $P < 0.05$.

	n	Ornithine decarboxylase activity (pmol of CO ₂ released/h per mg of protein)	Blood glucose (mg/dl)
Control			
Starved, 48 h	4	2 $\pm$ 0.6	56 $\pm$ 6
Refed, 3 h	4	11 $\pm$ 3*	57 $\pm$ 7
Diabetic			
Starved, 48 h	6	2 $\pm$ 0.3	94 $\pm$ 4
Refed, 3 h	4	5 $\pm$ 1	175 $\pm$ 28
Insulin, 3 h	4	41 $\pm$ 6*	4 $\pm$ 0.9
Refed + insulin, 3 h	5	26 $\pm$ 8*	79 $\pm$ 16

When insulin (0.2 unit/100 g body wt.) was administered to starved diabetic rats instead of food, muscle ornithine decarboxylase increased rapidly. However, the rats were profoundly hypoglycaemic, and the release of adrenaline and other counter-insulin hormones undoubtedly occurred. When the same amount of insulin was administered together with food to maintain normoglycaemia, skeletal-muscle ornithine decarboxylase increased 13-fold at 3 h to values even greater than in refed controls.

Discussion

The results indicate that polyamine metabolism is profoundly altered in insulin-deficient states. Thus in the present study we found marked decreases in ornithine decarboxylase activity in liver, heart, skeletal muscle and thymus of both starved and diabetic rats in association with a decrease in growth rate.

In rats studied 4 weeks after the induction of diabetes, Sochor *et al.* (1978) found diminished ornithine decarboxylase activity in liver, brain, heart and kidney, which could be prevented by insulin therapy. In contrast, Levine *et al.* (1978) have

reported a progressive increase in liver ornithine decarboxylase for up to 8 days after streptozotocin treatment in severely diabetic rats. Insulin therapy, as well as hypophysectomy and adrenalectomy, all prevented the increase in ornithine decarboxylase. Differences in the duration, severity and therapy of the diabetes, as well as the nutritional state of the animals, make comparison of these studies and our own difficult. In addition, in the previous studies and in the initial data presented here, ornithine decarboxylase was measured at a single point in time. This can lead to misleading information because diabetic rats may have a different eating pattern and because increases in ornithine decarboxylase activity may be transient. For these reasons, we investigated the effect of diabetes in an experimental model in which most of these variables could be controlled: the starved/refed rat.

The effect of starvation and refeeding on polyamine metabolism has been previously studied primarily in the liver and only for short time periods. Fausto (1969) was the first to observe a decrease in liver ornithine decarboxylase after an 18 h starvation period with an increase at 4 h after intubation of a casein hydrolysate. Domschke & Söling (1973) measured changes in ornithine decarboxylase and

putrescine during 5 h of refeeding after a 72 h starvation period. After refeeding, they found an increase in liver ornithine decarboxylase and putrescine with a peak in ornithine decarboxylase at 3 h. Besides these and other studies in liver (Hayashi *et al.*, 1972; Eloranta & Raina, 1977), Maudsley *et al.* (1976) observed an increase in ornithine decarboxylase in the small intestine of a 24 h-starved rat during refeeding, with peak stimulation 3–5 h after presentation of food.

In the present study, the effect of starvation and refeeding up to 96 h on ornithine decarboxylase activity was determined in liver, skeletal muscle, heart and thymus of normal and diabetic rats. Starvation for 48 h caused a 70–90% decrease in ornithine decarboxylase in all these tissues and eliminated the differences between control and diabetic rats. Refeeding caused a biphasic increase in hepatic ornithine decarboxylase in control rats. This biphasic response of ornithine decarboxylase in liver is not unique to the refeeding stimulus and has been shown to occur during the prereplicative period in liver stimulated to synthesize DNA by partial hepatectomy, the administration of a mixture of thyroxine, amino acids, glucagon and heparin, and cyclical ingestion of a protein-free/protein diet (Gaza *et al.*, 1973*b*). The first peak has been studied extensively and is generally accepted to be somatotropin-dependent, possibly synergistic with glucocorticoids (Russell & Synder, 1969; Hayashi *et al.*, 1972). It is found to occur 3–4 h after the stimulus and it is independent of the age of the animal (Hölttä & Jänne, 1972). The second peak has been less well characterized. Its timing is reported to be age-dependent (Hölttä & Jänne, 1972), but it is not dependent on growth hormone or glucocorticoids (Russell & Synder, 1969; Hayashi *et al.*, 1972; Gaza *et al.*, 1973*b*).

In diabetic rats, refeeding causes an increase in liver ornithine decarboxylase at 3 h that was greater than that seen in normal rats. Ornithine decarboxylase then decreased and there was no second peak. In untreated diabetes, insulin concentrations are low, whereas somatotropin (Hansen & Johansen, 1970; Navalesi *et al.*, 1975) and glucagon (Unger & Orci, 1976) concentrations are increased. Possibly, this altered hormonal profile exaggerates the initial ornithine decarboxylase response to the nutritional load. With regard to the second peak, its conspicuous absence in livers of diabetic rats suggests it is dependent on insulin. Oka & Perry (1976) have observed a biphasic response of ornithine decarboxylase in cultured mammary tissues with the second peak at 12 h dependent on insulin and prolactin. It has been suggested that the second peak of ornithine decarboxylase is related to DNA synthesis both in liver after partial hepatectomy (Gaza *et al.*, 1973*a,b*) and in cell cultures

stimulated to proliferate with serum (Heby *et al.*, 1975; McCann *et al.*, 1975).

In contrast with liver, ornithine decarboxylase activity increased slowly after refeeding in muscle, heart and thymus, and only a single increase in ornithine decarboxylase was observed. In diabetic rats, the increase in ornithine decarboxylase in these tissues was blunted.

The weight of the thymus was diminished in control rats after 48 h of starvation. This is in keeping with previous reports that starvation causes involution of the thymus due to inhibition of cell division and emigration of lymphocytes (Atkins & Beaven, 1975). Thymic weight was also diminished in diabetic rats fed *ad libitum*. Interestingly, refeeding after starvation resulted in little change in the weight of the thymus in the non-diabetic group, but in diabetic rats, it was associated with a progressive decrease in thymic weight. The reason for this is not clear. The fact that after 48 h of refeeding ornithine decarboxylase activity, which is located predominantly in the lymphocytes of the thymus (Beaven & DeJong, 1973; Atkins & Beaven, 1975), had increased 4-fold per mg of protein in the diabetic group adds to the complexity of the explanation.

The findings in studies with meal-fed normal and diabetic rats indicate that insulin is required for the induction of ornithine decarboxylase in skeletal muscle after food ingestion. Insulin has previously been shown to increase ornithine decarboxylase in rat liver (Panko & Kenney, 1971) and small intestine (Maudsley *et al.*, 1976) when administered to intact rats. Pharmacological doses of insulin were used in these studies, however, and it is difficult to assess whether the increases in ornithine decarboxylase were secondary to alterations in other hormones. In support of this is the finding that insulin caused only a minor increase in hepatic ornithine decarboxylase in hypophysectomized rats (Richman *et al.*, 1971). Stimulation *in vitro* of ornithine decarboxylase by supraphysiological concentration of insulin has been demonstrated in the perfused rat liver (Mallette & Exton, 1973) and in a variety of cells in tissue culture (Aisbitt & Barry, 1973; Hogan *et al.*, 1974; Yamasaki & Ichihara, 1977). On the other hand, insulin did not prevent a decrease in ornithine decarboxylase in rat hearts perfused with Krebs–Henseleit solution (Antony *et al.*, 1976).

Whether insulin, or a factor dependent on insulin, modulates the increase in ornithine decarboxylase after food ingestion cannot be determined from the results presented here. However, studies *in vitro* from our laboratory (Conover *et al.*, 1979) suggest the latter possibility is more likely and that the responsible factor may be a somatomedin. Consistent with this view are studies that have demonstrated that circulating somatomedins are decreased in starvation and uncontrolled diabetes (Phillips &

Young, 1976a,b; Takano *et al.*, 1978; Baxter *et al.*, 1979) and that in diabetic rats their concentrations can be restored to normal by insulin (Phillips & Young, 1976b).

In conclusion, the data indicate that polyamine metabolism, as reflected by ornithine decarboxylase activity, is markedly altered in diabetes and starvation. The polyamines appear to play an important role in cellular growth; thus, impaired regulation of their formation may account for the diminished growth of many tissues in insulin-deficient states.

These studies were supported by a grant from the Kroc Foundation. C. A. C. was the recipient of a National Science Foundation fellowship. We thank Ms. Patricia Hatch for her superb skill in measuring putresine and Ms. Mary Tortora for her all-round excellence as a secretary.

References

- Aisbitt, R. P. G. & Barry, J. M. (1973) *Biochim. Biophys. Acta* **320**, 610–616
- Antony, P., Gibson, K. & Harris, P. (1976) *J. Mol. Cell. Cardiol.* **8**, 589–597
- Atkins, F. L. & Beaven, M. A. (1975) *Biochem. Pharmacol.* **24**, 763–768
- Baxter, R. C., Brown, A. S. & Turtle, J. R. (1979) *Horm. Metab. Res.* **11**, 216–220
- Beaven, M. A. & DeJong, W. (1973) *Biochem. Pharmacol.* **22**, 257–265
- Booth, D. A. (1972) *J. Comp. Physiol. Psychol.* **80**, 238–249
- Conover, C., Rozovski, S. J. & Ruderman, N. B. (1979) *Clin. Res.* **27**, 592A
- Domschke, S. & Söling, H. D. (1973) *Horm. Metab. Res.* **5**, 97–101
- Dunnett, C. W. (1955) *J. Am. Stat. Assoc.* **50**, 1096–1121
- Eloranta, T. O. & Raina, A. M. (1977) *Biochem. J.* **168**, 179–185
- Fausto, N. (1969) *Biochim. Biophys. Acta* **190**, 193–201
- Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975) *Biochim. Biophys. Acta* **414**, 71–84
- Gaza, D. J., Short, J. & Lieberman, J. (1973a) *Biochem. Biophys. Res. Commun.* **54**, 1483–1488
- Gaza, D. J., Short, J. & Lieberman, I. (1973b) *FEBS Lett.* **32**, 251–253
- Hansen, A. P. & Johansen, K. (1970) *Diabetologia* **6**, 27–33
- Hayashi, S., Aramaki, Y. & Naguchi, T. (1972) *Biochem. Biophys. Res. Commun.* **46**, 795–800
- Heby, O., Marton, L. J., Zardi, L., Russell, D. H. & Baserga, R. (1975) *Exp. Cell Res.* **90**, 8–14
- Hogan, B. L. M., McIlhinney, A. & Murden, S. (1974) *J. Cell. Physiol.* **83**, 353–358
- Hölttä, E. & Jänne, J. (1972) *FEBS Lett.* **23**, 117–121
- Hopkins, H. A., Bonney, R. J., Walter, P. R., Yager, J. D. & Potter, V. R. (1972) *Adv. Enzyme Regul.* **11**, 169–191
- Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
- Levine, A. S. & Hegarty, P. V. J. (1977) *Nutr. Rep. Int.* **15**, 367–372
- Levine, J. H., Buse, M., Leaming, A. B. & Raskin, P. (1978) *Endocrinology* **103**, 1234–1238
- Li, J. B., Higgins, J. E. & Jefferson, L. S. (1979) *Am. J. Physiol.* **236**, 222–228
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mallette, L. E. & Exton, J. H. (1973) *Endocrinology* **93**, 640–644
- Marton, L. J., Heby, O. & Wilson, C. B. (1974) *FEBS Lett.* **46**, 305–307
- Maudsley, D. V., Leif, J. & Kobayashi, Y. (1976) *Am. J. Physiol.* **231**, 1557–1561
- McAnulty, P. A. & Williams, J. P. G. (1977) *Biochem. J.* **162**, 109–121
- McCann, P. P., Tardif, C., Mamont, P. S. & Schuber, F. (1975) *Biochem. Biophys. Res. Commun.* **64**, 336–341
- Nakano, K. (1978) *Nutr. Rep. Int.* **18**, 454–464
- Navalesi, R., Pilo, A. & Vigneri, R. (1975) *Diabetes* **24**, 317–327
- Oka, T. & Perry, J. W. (1976) *J. Biol. Chem.* **251**, 1738–1744
- Panko, W. B. & Kenney, F. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 346–350
- Phillips, L. S. & Young, H. S. (1976a) *Endocrinology* **99**, 304–314
- Phillips, L. S. & Young, H. S. (1976b) *Diabetes* **25**, 516–527
- Richman, R. A., Underwood, L. E., VanWyk, J. J. & Vaina, S. L. (1971) *Proc. Soc. Exp. Biol. Med.* **138**, 880–884
- Rozovski, S. J., Rosso, P. & Winick, M. (1978) *J. Nutr.* **108**, 1680–1690
- Rozovski, S. J., Conover, C. A. & Ruderman, N. B. (1979) *Life Sci.* **25**, 553–560
- Russell, D. H. & Synder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
- Russell, D. H. & Synder, S. H. (1969) *Endocrinology* **84**, 223–228
- Sochor, M., Baquer, N. Z., Hothersall, J. S. & McLean, P. (1978) *Biochem. Biophys. Res. Commun.* **80**, 533–539
- Takano, K., Hizuka, N., Kawai, K. & Shizume, K. (1978) *Acta Endocrinol. (Copenhagen)* **87**, 485–494
- Unger, R. H. & Orci, L. (1976) *Physiol. Rev.* **56**, 778–826
- Wool, I. G., Strewall, W. S. & Mayer, A. N. (1968) *Am. J. Physiol.* **214**, 825–831
- Yamasaki, Y. & Ichihara, A. (1977) *J. Biochem. (Tokyo)* **81**, 461–465