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Effect of Alloxan-diabetic Rat Fed with Different Diets on Ureogenesis in Isolated Perfused Liver

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The effect of alloxan-diabetic rat fed with normal, high fat, low protein and high protein diets on the rate of urea production and the activities of enzymes associated with the urea cycle (ornithine transcarbamoylase, E.C. 2.1.3.3, OTC; arginase, E.C. 3.5.5.1) have been studied in intact and isolated perfused liver.

The amount of urea excretion was the highest in the high protein diet group. When each diet group was treated with alloxan, total urea excretion showed little differences between each diet group and its corresponding control group with the exception being in the normal diet group.

However, the enzyme activity of OTC was increased significantly by alloxan treatment in low and high protein diet groups as compared to corresponding control groups. Similar results were obtained in arginase activity, although the magnitude of the change was less marked.

In liver perfusion experiments on rats treated with alloxan, the amount of urea production and changes in OTC and arginase activity were very similar with those in the intact liver.

These results suggest that alloxan treatment in normal diet group causes an increase in urea excretion both in intact and perfused liver regardless of changes in enzyme activities and total urea excretion, and enzyme activities are affected by changes in dietary components but the changes of enzyme activities may not correlate with total urea excretion

Key Words: Alloxan-diabetes, Different diet, Ureogenesis

Since the mechanism for urea formation from NH_3 and CO_2 was proposed (Krebs and Henseleit, 1932), much research on the controlling factors that regulate urea synthesis have been undertaken. According to previous studies, the activities of enzymes involved in the urea cycle increased during starvation, by ingestion of high protein diet or by corticosteroid treatment (Freedland & Sodikoff, 1962., Schimke, 1962b., Das & Waterlaw, 1974., Mclean & Gureny, 1963) and the rate of urea synthesis

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depended on the activity of enzymes of the urea cycle (Schimke, 1962a) and the concentrations of ornithine and N-acetylglutamate (Saheki et al, 1977., Briggs & Freedland, 1977) in the liver. Schimke (1963) reported that the concentrations of liver ornithine, citrulline, and arginine maintained the steady state regardless of the amount of excreted urea, but the amount of enzymes involved in the urea cycle was influenced by diet intake, starvation, hormone and amino acids. Therefore, he concluded the activities of the urea cycle enzymes would be major controlling factors. Further more, Stephen (1968) also reported that the urea cycle enzymes were regulated by protein diets. Das & Waterlaw (1974) and Hayase *et al* (1980) showed that there was an increase in urinary urea excretion without a comparable increase in the amount of enzyme when the diet containing high quality protein was replaced by the isonitrogenous diet with low quality protein, indicating that enzyme activities would not be controlling factors, but the degree of availability of substrate would be major controlling factors in the rate of urea synthesis. It was demonstrated that when the substrates necessary for urea synthesis were present in excess, the addition of a metabolic intermediate of the urea cycle caused increased synthesis of urea in vivo (Greenstein *et al*, 1956) and in the liver perfusion system (Kramer & Freedland, 1972., Hems *et al* 1966), and also in the isolated hepatocyte experiment (Briggs & Freedland, 1976). Therefore, they proposed that the rate of urea synthesis depends primarily on substrate and urea cycle intermediate, rather than enzyme activity (Briggs & Freedland, 1977., Hayase *et al*, 1980). The two hypotheses are controversial: the urea biosynthesis depends on the enzyme activities of the urea cycle, and the urea biosynthesis depends on the substrate and urea intermediate concentration. This report presents studies on the relationship between the amount of urinary urea excretion and the activities of urea cycle enzyme in the rats fed various diets, and in the isolated liver perfusion system, how the amount of urea excretion and the activities of urea cycle enzymes were influenced by alloxan treatment in rats fed different diets.

MATERIAL AND METHODS

Apparatus and Reagents

Microburette was pruchased from Manostat Corporation, Conway dishes from Brunswick Laboratory, and liver perfusion apparatus from Metalloglass Inc, Co. (USA). Ornithine, arginine, urea, and carbamoyl phosphate were purchased from Sigma Chem. Co, alloxan, urease, ethyleneglycol were from Merck Co, and antipyrine diacetylnmonoxime from Kwan Dong Chemical Co. (Japan), p-dimethylaminobenzaldehyde from Smith, New York.

Animals

Male rats weighing about 120g were used in all experiments. Rats were divided into normal, high fat, low protein and high protein diet groups, and fed that diet for 14 days. Diet compositions were based on the modified method of DeCarli & Lieber (1967). Normal diet (protein 18%, carbohydrate 47%, fat 35%), high fat diet (protein 18%, carbohydrate 5%, fat 77%), and low protein diet (protein 5%, carbohydrate 60%, fat 35%) contained appropriate amounts of minerals and vitamins. Designate diet and tap water supplied ad libitum.

Urea excretion and enzyme activities in vivo.

Each diet group was divided into a control and a test group and alloxan (200mg/Kg BW in 0.9% saline) was injected in once intraperitoneally in to the test group and 1 ml of saline was injected in the control group and maintained

for 2 weeks in the metabolic cages. During the maintenance of each diet group, urines were collected everyday and determined the amount of urea by the microdiffusion method (Obrink 1955). After 14 days feeding on the disignated diet, rats were decapitated, blood was collected, the liver was removed and weighed immediately. Glucose concentration was determined by the Folin-Wu method (1929), ornithine transcarbamoylase activities by the method of Cerotie & Gazzaniga (1966), and arginase activities by Ehrlich's reagents of Kaysen & Strecker (1973).

Urea production and enzyme activities in the isolated liver perfusion system.

Perfusion of the isolated rat liver of each diet group was carried out according to the technique of Miller *et al*, (1951). The rats were anesthetized for operation by ether inhalation. The livers were rapidly removed and perfusion was carried out at 37C. The perfusate contained 30ml beef RBC, 1.5g of bovine serum albumin in 80ml of Ringer solution. The pH of the perfusate was adjusted to 7.4 automatically by 0.2M bicarbonate solution. Heparin (1000unit) was added to the perfusate and continuously recycled and was equilibrated with a mixture consisting of 95% O_2 and 5% CO_2. Under these conditions, perfusion was performed for 6 hours and 0.5ml of perfusate was taken every hour for measurement of urea production. After perfusion, OTC and arginase activities were assayed in the liver. Protein was determined by the Lowry *et al*, method (1951).

RESULTS

1) Urinary urea nitrogen in rats fed various diets.

Urinary urea nitrogen was measured daily during the 14 days maintenance. The results are shown in Fig. 1. Urea excretion was the highest in the high protein diet group and in the normal diet, high fat diet, low protein diet group in decreasing order. When alloxan was administered to each diet group, the total amount of urea excreted showed little change compared with the corresponding control group in high protein, low protein, and high fat diet group, but increased significantly in the normal diet group treated with alloxan (4694±185mg/14 day) as compared to its control group (3073± 160mg/14day). These results indicate that the urinary urea excretion was dependant on diet composition, but urea excretion was not influenced by alloxan treatment except in the normal diet group.

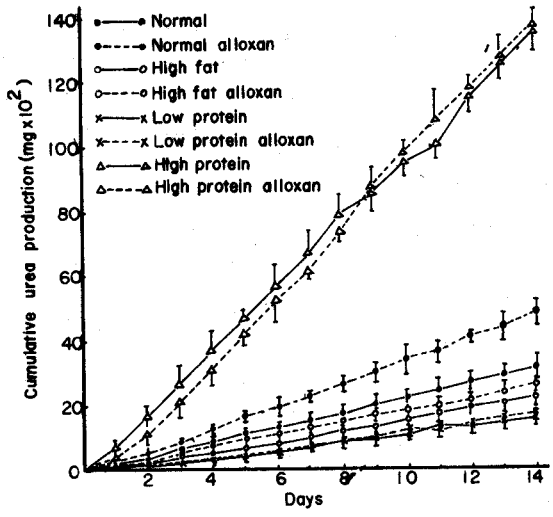

Fig. 1. Effect of alloxan induced diabetic rat in different diet group on urea production.

2) The effect of diet composition and alloxan treatment on the activity of ornithine transcarbamoylase (OTC) and arginase in the rat liver.

Table 1 shows the effect of alloxan on the activity of OTC and arginase, OTC activity of alloxan treated normal diet group (9.71±1.22n moles/mg of protein) was decreased as compared to its control group (15.33±0.41), but no significant changes observed between alloxan treated

Table 1. Effect of alloxan-diabetes on the activity of ornithine transcarbamoylase and arginase on the liver of rats on different diets

Treatment / Exp. group	Ornithine transcarbamoylase (n moles/mg of protein)		Arginase (mg/mg of protein)	
	Control	Alloxan	Control	Alloxan
Normal (3)*	15.33±0.41	9.71±1.22	0.464±0.04	0.432±0.04
High fat (4)	10.33±2.16	8.87±1.05	0.374±0.01	0.571±0.07
Low protein (3)	5.47±1.79	8.18±1.47	0.357±0.03	0.500±0.07
High protein (5)	10.08±2.26	19.22±1.80	0.498±0.02	0.635±0.08

* No. of rat

** mean±S.E.

high fat diet (8.87±1.05) and its control group (10.33±2.16). However, the enzyme activity of OTC increased significantly in the alloxan treated group of low and high protein diet group (5.47±1.79, 10.08±2.26). The arginase activity in the alloxan treated high fat diet (0.571±0.07 mg/mg og protein), low protein diet (0.500± 0.07), and high protein diet (0.635±0.08) increased significantly as compared to their control groups of high fat diet (0.374±0.01). low protein diet (0.357±0.03), high preotein diet (0.498±0.02), but no significant difference was found in the alloxan treated normal diet group (0.432±0.04mg/mg of protein) compared to its control group (0.464±0.04). Our results suggest that when rats were fed a normal diet, the activities of arginase were not affected by alloxan, but when diet compositions were changed, arginase activity was influenced by alloxan treatment. These results (Figure 1. and Table 1) indicate that the enzyme activities concerned with the urea cycle does not correlate with urea production.

3) Effect of diet composition on blood glucose.

The concentration of blood glucose in the diet control and corresponding alloxan treated diet groups is shown in table 2. Each diet group of

Table 2. Glucose concentration in blood of alloxan diabetic and its control rat in different diet group

Treatment / Exp. group	Glucose concentration (mg%)	
	Control	Alloxan
Normal (3)*	153±17.0**	353±17.6
High fat (4)	157±5.8	321±10.1
Low protein (3)	139±5.9	320±20.4
High protein (5)	188±26.3	326±2.6

* No. of rat

** mean±S.E.

alloxan treated showed a two fold or more increase in blood glucose over its corresponding control group. These results demonstrated that the alloxan diabetic rat was induced by one dose of alloxan.

4) Urea production and enzyme activity in isolated perfusion experiment.

The amount of urea from the perfusate in alloxan treated and control group fed different diet was measured. In the high protein diet group, the excreted urea in alloxan treated group was 54.3mg% and 42.2mg% in its control group. In the normal and high fat diet groups, alloxan treated groups were 28.7 and 26.5mg%, and their control groups were 19.5 and 18.4mg%. In the low protein diet group, alloxan treated

Table 3. Effect of alloxan-diabetes on the ornithine transcarbamoylase activity in the liver perfusion of rats on different diets.

Exp. group \ Treatment / Time (hr)		Ornithine transcarbamoylase activity (n moles/mg of protein)	
		Control	Alloxan
Normal (3)*	0	15.33±0.41**	9.71±1.22
	6	14.70±1.77	10.19±2.05
High Fat (4)	0	10.33±2.16	8.87±1.05
	6	10.37±1.55	7.50±2.40
Low protein (3)	0	5.47±0.79	8.18±1.47
	6	3.27±0.87	9.47±0.73
High protein (5)	0	10.08±2.26	19.22±1.80
	6	7.81±1.05	14.57±1.45

* No. of rat

** mean±S.E

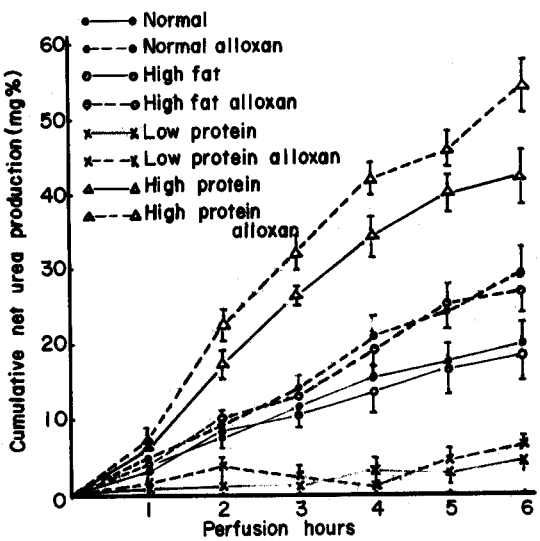

Fig. 2. Urea production by isolated rat liver in the perfusion system.

Table 4. Effect of alloxan-diabetes on the arginase activity in the liver perfusion of rats on different diets.

Exp. group \ Treatment / Time (hr)		Arginase activity (mg/mg of protein)	
		Control	Alloxan
Normal (3)*	0	0.464±0.04**	0.432±0.04
	6	0.518±0.07	0.560±0.05
High fat (4)	0	0.374±0.01	0.571±0.07
	6	0.478±0.05	0.569±0.06
Low protein (3)	0	0.357±0.03	0.500±0.07
	6	0.391±0.01	0.502±0.06
High protein (5)	0	0.498±0.02	0.635±0.08
	6	0.548±0.03	0.622±0.09

* No. of rat

** mean±S.E.

group was 6.2mg%, and 4.5mg% in its control group. These results indicated that alloxan increased urea production in normal, high fat and high protein diet groups, but not in the low protein diet group. OTC activities in each group were shown in Table 3. In normal diet, OTC activity in the alloxan treated group (10.19±2.05n moles/mg of protein) was lower than its control group (14.70±1.77). In the high fat diet group, OTC activity in the alloxan treated group (7.50±2.40) were similar to its control group (10.37±1.55). However, in the low protein and high protein diet groups, OTC activity in the alloxan treated groups (9.47±

0.73, 14.57±1.45) was increased two or more fold compared to their control groups (3.27± 0.87, 7.81±1.05). The arginase activity was similar in the normal, high fat and high protein groups treated with alloxan (0.560±0.05, 0.569± 0.06 and 0.622±0.09) with respect to their control groups (0.518±0.07, 0.478±0.05 and 0.548± 0.03). But the alloxan treated group in the low protein diet group (0.502±0.06) showed 30% increase in enzyme activities as compared to its control (0.391±0.01) group. These results showed that effect of different diet and alloxan treatment on urea production, OTC and arginase activities in intact experments were similar to the isolated perfused liver experiment (Figure 2, Table 3,4), which indicates urea production does not correlated to the enzyme activities concerned in the urea cycle, but it depends on the diet composition.

DISCUSSION

It has been demonstrated that enzyme activities concerned with the urea cycle were affected by hormones and diet (Freedland *et al*, 1968) and again demonstrated the complexity between urea cycle enzymes and the substrate (Kramer & Freedland, 1972). Schimke (1962a) reported that the increase of dietary protein caused proportional increase in enzyme activity, especially OTC and arginase, and in the urea synthesis in rats. However, Ashida and Harper (1961) have shown that the urea cycle enzyme levels were not proportional to urinary urea production. As shown in Fig. 1, the amount of urea excretion was the highest in high protein diet group and in the normal diet, high fat diet, low protein diet group in decreasing order, and the alloxan treatment in the normal diet group only increased the urea excretion by 50% as compared to its control group. These results indicate that urea excretion is influenced by dietary composition and external factors such as insulin, which coincide with the findings in diabetic patients. Diabetic patients have been known to excrete increased amounts of urea and nitrogen (Soskin & Levine, 1946., ChaiKoff & Forker, 1950). Glucagon which is concerned in protein catabolism increases urea synthesis (Tyberghein, 1953., Miller, 1960). Because insulin and glucagon have potent antagonistic action on carbohydrate and nitrogen metabolism, it is easily understandable that urea production is increased in alloxan-diabetes (Zaleski and Bryla, 1978). According to Das and Waterlaw (1974), animals have high capacities of enzymes involved in urea cycle, and urea production can be increased without changing enzyme activities. However, Hayase *et al*, (1980) reported that a diet containing high quality protein induced arginase activity and reduced the urea excetion, but diet with low quality protein had the opposite effect. Therefore, it seems likely that arginase activity and urea production have an inverse relationship. It has been shown that some intracellular amino acids regulated the urea production by controlling the enzyme concered with the urea cycle. As high concentration of intracellular alanine suppresses OTC and argininosuccinate synthetase, urea synthesis is decreased (Wojtczak *et al*, 1978). Isoleucine, valine, lysine, and leucine suppress urea production by inhibiting arginase or argininosuccinate synthetase (Kaysen & Strecker, 1973). These facts indicate that amino acid composition in diet plays an important role on the urea production and urea cycle enzymes. As shown in table 1, in normal diet group, alloxan treatment decreased OTC activity as compared to the control group, but in the high fat diet group, there's no difference in either and in the low protein and high protein groups, alloxan administration led to an increase in the OTC activity, indicating that the hormonal factor (insulin) also

plays an important role on the urea production and urea cycle enzymes (Table 1, 3, 4). The activity of arginase in the normal diet group was not changed by the alloxan treatment, but in the high fat, low protein and high protein diet groups, arginase activity was increased similarly to OTC activity. Our results suggest that the alter ations of enzyme activity would be due to the imbalance of amino acids in diets. In the perfusion experiment, the urea production and OTC and arginase activities in different diet groups are similar to the results found in vivo. (Figure 1, 2, Table 1, 3, 4). From these results, we can conclude that urea production and enzyme activity concerned with urea synthesis is not correlated directly to each other, but urea production depends probably on the amino acid composition in the diet and an other external hormonal factors (insulin).

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