Effects of Thiamine Deficiency and Octanoate Administration, *in Vivo*, on Gluconeogenesis in Rat Kidney Slices and on Amino Acid Profile in Rat Liver (36583)

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Previous studies with rats have suggested that thiamine deficiency may produce an impairment of gluconeogenesis. In 1941, Barron *et al.* (1) reported that kidney slices from thiamine-deficient rats, utilizing pyruvate as substrate, synthesized glucose and tricarboxylic acid cycle intermediates at a reduced rate, but that this effect could be reversed by addition of thiamine, *in vitro*. A subsequent study (2, 3) reported that mitochondria from livers of thiamine-deficient rats carboxylated pyruvate at a reduced rate but that fatty acids and acylcarnitines, *in vitro*, reversed the effect.

The present experiments were conducted to study in more detail the interrelationships between thiamine deficiency and gluconeogenesis. The effect of thiamine deficiency on glucose synthesis by kidney slices was determined in the presence and absence of octanoate, which is a known stimulator of gluconeogenesis (4, 5). In addition, the effect of *in vivo* octanoate on amino acid profile in liver was determined and discussed in relationship to altered rates of gluconeogenesis.

Methods. Animals. Male rats (CFE strain, Carworth Farms, Portage, MI) weighing between 85 and 100 g were paired by weight, kept in individual cages with 0.5 in. wire-mesh bottoms to prevent coprophagy, and pair-fed a thiamine-deficient diet. The diet composition and technique of pair-feeding have been described (2). The control rats received 0.6 mg of thiamine/kg/day, orally. The deficient rats and their partners were used only after extreme weight loss and severe ataxia (indicative of the terminal stage of deficiency) were apparent. The terminal stage developed after 30–35 days on the deficient diet.

Octanoate administration. Four thiamine-deficient rats and their partners received a single intraperitoneal injection of 3.7 mmoles octanoate/kg, 3 hr prior to sacrifice. A second group of deficient and control rats received similar volumes of physiological saline. The octanoate caused a slight debilitation lasting for approximately 20 min. At 3 hr after octanoate administration there was no visible difference between those rats which had received octanoate and those which had not.

Kidney slices. Kidneys were removed and placed in ice-cold Krebs–Henseleit bicarbonate buffer, pH 7.3. Cortex slices were prepared and incubated by previously described procedures (6). After incubation, the slices were removed from the flasks, dried overnight at 110° and weighed. The wet wt:dry wt ratio was 5.3 for both the thiamine-deficient and the pair-fed control rats. Glucose, in the medium, was determined by a modified glucose oxidase method (6).

Amino acids. Livers were extirpated and homogenized immediately in 1% picric acid. Samples were prepared for amino acid analysis by the method of Spackman (7). Amino acids were determined with a Beckman 116 amino acid analyzer. Acidic and neutral amino acids required the use of a lithium buffer system to facilitate the separation of glutamine and asparagine (8). Basic amino acids were analyzed by the manufacturer's suggested method for amino acids in biological fluids.

Results and Discussion. The synthesis of

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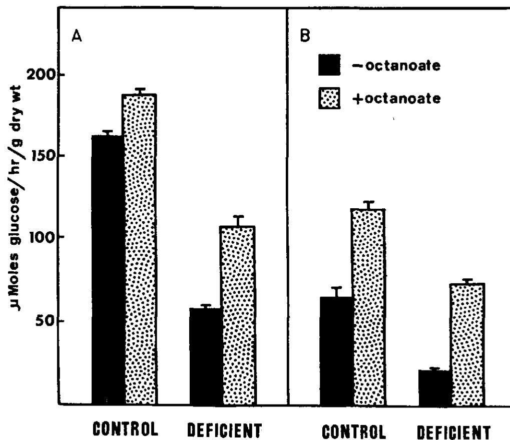

FIG. 1. The effect of octanoate on glucose synthesis by rat kidney cortex slices from thiamine-deficient and pair-fed control rats. (A) Substrate: 10 m*M* pyruvate; (B) substrate: 10 m*M* lactate. Slices were prepared an incubated by the method of Krebs *et al.* (6). Octanoate was administered intraperitoneally, at 3.7 mmoles/kg, 3 hr prior to sacrifice. Each value represents the mean, obtained from 4 rats, ± SEM. Each single rat value was the mean of several replicate incubations.

glucose by kidney cortex slices from pair-fed control and thiamine-deficient rats is shown in Fig. 1. When either pyruvate or lactate was utilized as the gluconeogenic substrate, the rate of glucose synthesis was significantly lower in slices from the kidneys of the thiamine-deficient rats. The *in vivo* administration of octanoate greatly increased the rate of glucose synthesis in slices from both deficient and control rats but, on a percentage basis, the increase was greater with the deficient slices. These results suggest that gluconeogenesis may be depressed in thiamine-deficient rats but show that the actual rate as determined *in vitro* can be regulated by the availability of fatty acids such as octanoate. It must, therefore, be assumed that there is no biochemical lesion in thiamine deficiency which inexorably results in decreased gluconeogenesis. Rather, it is most probable that gluconeogenesis was affected indirectly via a metabolic inhibition which could be circumvented if adequate fatty acid was available. The experiments of Paquet *et al.* (2) and Mehlman *et al.* (3) with rat liver mitochondria showed that pyruvate oxidation and carboxylation were inhibited by thiamine deficiency but that the carboxylation, a key reaction in gluconeogenesis, could be greatly in-

TABLE I. Effects of Intraperitoneal Administration of Octanoate on the Concentrations of Amino Acids in Livers of Thiamine-Deficient and Pair-Fed Control Rats (μmoles Free Amino Acid per 100 g body wt ± SEM).[a]

	Rat treatment			
	Pair-fed (4)		Thiamine-deficient (4)	
Amino acid	Without octanoate (A)	With octanoate (B)	Without octanoate (C)	With octanoate (D)
Aspartate	2.63 ± 0.3	1.08 ± 0.07[d]	1.74 ± 0.21[e]	1.23 ± 0.06
Glutamate	7.18 ± 0.52	2.91 ± 0.01[d]	9.79 ± 0.86	6.27 ± 0.97[b]
Glutamine	9.85 ± 1.3	4.09 ± 0.71[c]	10.21 ± 1.14	5.41 ± 0.67[c]
Serine	1.22 ± 0.13	0.56 ± 0.12[c]	1.36 ± 0.03	0.71 ± 0.12[d]
Threonine	0.72 ± 0.007	0.56 ± 0.12	1.23 ± 0.10[f]	0.92 ± 0.06
Glycine	5.45 ± 0.49	5.32 ± 0.96	6.45 ± 0.49	6.80 ± 1.18
Alanine	5.91 ± 0.44	4.19 ± 0.29[c]	6.17 ± 0.43	5.47 ± 0.61

[a] The liver wt:body wt ratio was 0.035 ± 0.001 for the thiamine-deficient and 0.027 ± 0.001 for the pair-fed control rats. The number of animals per group is shown in parentheses.

[b] $p < .05$ for A vs B and C vs D.

[c] $p < .025$ for A vs B and C vs D.

[d] $p < .01$ for A vs B and C vs D.

[e] $p < .05$ for A vs C.

[f] $p < .01$ for A vs C.

creased by octanoate, *in vitro*. Most probably, thiamine deficiency caused a direct inhibition of pyruvate dehydrogenase, the effects of which were minimized by the presence of an alternative source of energy for ATP synthesis such as fatty acid.

In vivo administration of octanoate causes an increased rate of gluconeogenesis which can be correlated with decreased levels of gluconeogenic amino acids in liver (4). Table I shows liver amino acid profiles of thiamine-deficient and pair-fed rats and the effects of octanoate on these profiles. Thiamine deficiency caused alterations in only two of the measured amino acids, threonine was increased and aspartate decreased. Administration of octanoate resulted in decreased concentrations of amino acids in livers from both deficient and control rats. Glycine concentrations were not altered in response to octanoate.

Our results suggest that thiamine deficiency produced an indirect inhibition of gluconeogenesis and that gluconeogenesis in both normal and deficient rats was sensitive to regulation by fatty acids.

Summary. The effects of a single dose of octanoate (3.7 mmoles/kg, ip) on gluconeogenesis in kidney slices and on amino acid profile in liver were determined in thiamine-deficient and pair-fed control rats. Kidney slices from the deficient rats produced glucose, from lactate or pyruvate, at greatly reduced rates. Octanoate, *in vivo,* increased the rates of glucose synthesis in slices from both control and deficient rats.

Thiamine deficiency caused increased levels of threonine and decreased levels of aspartate but glutamate, glutamine, serine, glycine and alanine were unchanged. Octanoate administration, *in vivo,* to both control and diabetic rats, reduced the concentrations of all liver amino acids, except glycine.

It was concluded that thiamine-deficiency inhibited gluconeogenesis indirectly, possibly via a primary lesion at pyruvate dehydrogenase, but that the inhibition could be circumvented by providing a fatty acid (*e.g.*, octanoate).

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