Alanine and Glutamine Levels in Rat Liver Tissue: A Direct Relationship to Gluconeogenic State[1] (34195)

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The metabolic pathway of gluconeogenesis has been studied extensively (1) and in mammals it occurs prominently in liver and kidney cortex (2). The process provides the mammalian organism a means of producing glucose from noncarbohydrate precursors. It is enhanced in starvation or when the diet is devoid of carbohydrate and depressed when carbohydrate supply is abundant. Mobilization of tissue amino acids represents a prominent source of noncarbohydrate precursors for gluconeogenesis. The present experiments were performed to compare concentration changes of individual amino acids in liver, a tissue exhibiting gluconeogenesis, and in skeletal muscle, a tissue with predominantly a glycolytic pathway. In addition, free amino acids were determined in blood plasma so that intratissue transport could be evaluated. Fasting and carbohydrate feeding represented conditions to augment and depress gluconeogenesis, respectively. Experiments were carried out also to examine the effect of the administration of octanoate which has been reported to enhance gluconeogenesis (3, 4).

Material and Methods. Adult male rats of the Wistar strain, reared in our laboratory, weighing 280–350 g were fed a control diet described previously (5) for 7 days before experiments were undertaken. Animals were housed in individual cages and unless indicated otherwise were allowed distilled water *ad libitum*. Six rats were fed a high carbohydrate diet (glucose replacing the casein of the control diet isocalorically) for 24 hr. Six rats were fasted 24 hr. Three rats were fed the high-carbohydrate diet 24 hr and 2 hr before ending the experiment each animal received 360 μmole sodium octanoate/100 g of body weight by intraperitoneal injection. Three rats were fasted 24 hr, and 2 hr before ending the experiment each animal received sodium octanoate.

At completion of an experimental period, rats were placed under sodium pentobarbital anesthesia. Arterial blood collected by cardiac puncture was placed in an heparinized tube and kept in ice until centrifuged in a refrigerated centrifuge to obtain plasma for analysis. Liver tissue was excised promptly and placed in a pocket of dry ice. Skeletal muscle (gastrocnemius) then was collected and similarly placed in a pocket of dry ice. Chemical analyses were initiated immediately after collection of samples. For the determination of free amino acids, picric acid filtrates of plasma, liver, and skeletal muscle were prepared and excess picric acid removed according to Stein and Moore (6) and Tallen *et al.* (7). Aliquots of picric acid free filtrates were dinitrophenylated and the resulting derivatized material was fractionated by silica gel column chromatography (8), with slight modification (9).

Results and Discussion. The concentration of non-essential amino acids in liver was higher in all animals consuming carbohydrate. When abrupt changes in the gluconeogenic state were initiated, it was the nonessential amino acids again which were primarily involved. The most conspicuous and consistent

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TABLE I. Tissue Amino Acid Levels under Conditions of Altered Gluconeogenesis.

Group	Gluconeogenic state	Amino acid	Tissue concentration[a] Liver	Muscle	Plasma
24-hr fast plus octanoate	↑ ↑	Ala	88.8 ± 22	204 ± 19	109 ± 9.9
		Glu NH_2	684 ± 111	404 ± 89	290 ± 9.2
24 hr fast	↑	Ala	181 ± 56	237 ± 40	132 ± 23
		Glu NH_2	846 ± 332	378 ± 43	290 ± 31
High carbohydrate diet plus octanoate	↓ ↑	Ala	306 ± 23	293 ± 17	122 ± 4.9
		Glu NH_2	1450 ± 95	546 ± 13	280 ± 7.9
High carbohydrate diet	↓	Ala	661 ± 135	326 ± 36	214 ± 27
		Glu NH_2	2340 ± 592	528 ± 23	316 ± 10

[a] Mean values: μmoles/100 g of fresh liver and muscle; and μmoles/100 g of plasma water ± SD.

changes, however, were found in the concentrations of alanine and glutamine in the liver. Table I lists a comparison of the tissue concentrations of these two amino acids under the experimental conditions described. Under conditions of depressed gluconeogenesis, the concentrations of these amino acids in liver were markedly increased whereas under conditions of enhanced gluconeogenesis their concentrations were markedly decreased. Although similar changes of much smaller magnitude were observed in muscle for alanine concentration, glutamine levels did not follow any consistent pattern. The changes in muscle and plasma presumably are a reflection of mobilization to the liver.

These results are in harmony with the observations of Felig *et al.* (10), who found marked extraction of alanine by the human liver in the absence of any significant change in arterial alanine concentration. Because of the inherent difficulties noted by Adibi (11) in measuring glutamine by means of ion exchange procedures, the fluctuations in the concentration of this amino acid was not previously observed. The DNP–amino acid method used in this study (9) permits the separation of glutamine and asparagine as distinct peaks whereas the derivatization limits the losses due to spontaneous chemical transformations.

Summary. The free amino acid content of plasma, muscle, and liver from rats was compared under conditions of augmented and depressed gluconeogenesis. The concentration of alanine and glutamine in liver was consistently and markedly reduced during enhanced gluconeogenesis and increased during depressed gluconeogenesis.

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