Histidase Expression Is Regulated by Dietary Protein at the Pretranslational Level in Rat Liver\textsuperscript{1,2}

Nimbe Torres,\textsuperscript{3} Laura Martínez, Gabriela Alemán, Héctor Bourges and Armando R. Tovar

Departamento de Fisiología de la Nutrición, Instituto Nacional de la Nutrición “Salvador Zubiran,” México, D. F. 14000, México

ABSTRACT The effect of dietary protein on the expression of histidase (Hal) was investigated to understand the mechanism of induction of histidase by a high protein diet. In this study, we examined the following: (1) the effect of 0, 6, 18, and 35 and 50% casein diets on hepatic and epidermal Hal activity, amount of the enzyme and Hal-mRNA; (2) the effect of a high histidine diet (1.25%) on Hal expression; (3) the response of Hal expression in rats fed a 10% casein diet and injected with glucagon (0.6 mg/(100 g body wt · d); and (4) the half-lives of the enzyme and Hal-mRNA in rats fed an 80% casein diet for 7 d followed by a protein-free diet. Hal activity increased as the protein content in the diet increased ($r = 0.986, P < 0.001$) and was associated with a significant increase in $V_{\text{max}}$ without a change in $K_m$. The dietary regulation was liver specific because skin Hal was unresponsive. Increments in hepatic Hal activity were accompanied by concomitant significant increases in the amount of histidase and its mRNA. The response was more pronounced in rats fed diets containing >18% casein. Rats fed a 12% casein diet containing 1.25% histidine did not have different Hal activity and mRNA levels compared with rats fed a 12% casein diet, indicating that Hal expression is not modified by its substrate. Injection of glucagon into rats fed the 10% casein diet increased Hal activity threefold and Hal-mRNA expression fivefold compared with un.injected rats fed the same diet. The apparent half-life of hepatic histidase in protein-depleted rats previously fed an 80% casein diet was 2.8 d, whereas the half-life of Hal-mRNA was 17 h. In summary, these data support the hypothesis that Hal expression is regulated by dietary protein at the pretranslational level in rat liver, and that glucagon is one of the hormones involved in the induction of Hal. J. Nutr. 128: 818–824, 1998.

KEY WORDS: • dietary protein • histidase • histidine • gene expression • rats

Dietary regulation of the gene expression of enzymes involved in the metabolism of carbohydrates and lipids has been extensively studied (Clarke and Abraham 1992, Hillgartner et al. 1995). However, few studies have been conducted to understand the expression of enzymes involved in the metabolism of amino acids. The amounts of most amino acid–degrading enzymes in the liver change in response to changes in protein intake, increasing as protein intake rises and decreasing as protein intake falls. A high protein intake does not lead to the accumulation of either body protein or unique storage proteins.

Histidase (Hal, histidine ammonia-lyase EC 4.3.1.3) is the first enzyme in the degradation of histidine. This cytoplasmic enzyme is widely distributed in vertebrates and catalyzes the irreversible nonoxidative deamination of L-histidine, generating urocanic acid and ammonia (Mehler and Tabor 1953, Peterkofsky 1962). Hal plays an important role in directing enzymes in the liver change in response to changes in liver and skin (La Du 1971).

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and Hal-mRNA expression are affected by the concentration of dietary protein or by a high histidine diet. We studied the effect of glucagon as a possible mediator in the regulation of histidase expression. It has been reported that glucagon increases after the ingestion of a protein meal (Charlton et al. 1996), and it is a potent stimulator of the activity of some amino acid–degrading enzymes (Jost et al. 1968, Lee and Harper 1971). However, there is no direct evidence concerning the mechanism of this reported effect. Therefore, we also studied the effect of glucagon as a possible mediator of Hal expression. Finally, this study was designed to assess how rapidly the Hal activity and the corresponding mRNA disappear from liver in rats switched from a high protein to a protein-free diet.

MATERIALS AND METHODS

Reagents and chemicals. Glucagon was purchased from Eli Lilly (Indianapolis, IN). Vitamin-free casein, mineral mix and vitamin mix were purchased from Teklad Test Diets, Madison, WI. Goat anti-rabbit immunoglobulin G (IgG) y-chain specific affinity purified antibody conjugated to horseradish peroxidase was obtained from KPL (Gaithersburg, MD); the nitrocellulose membranes were from Bio Rad (Hercules, CA). Nylon membrane filters (Hybond-N+), redi- prime DNA labeling system and deoxyctydine 5’[α-32P] triphosphate (110 TBg/mm2) were purchased from Amersham (Buckinghamshire, UK) and Gene Clean II kit from Bio 101 (La Jolla, CA).

Animals, diets and treatments. Male Wistar rats (80–90 g), ob-tained from the Experimental Research Department and Animal Care Facilities at the National Institute of Nutrition, Mexico D. F., were housed individually in wire stainless steal cages at 22°C with a 12-h light:dark cycle, and different groups of rats were used for one of the following four experimental protocols. The protocols used in these experiments were approved by the Animal Care Committee of the National Institute of Nutrition, México, D.F.

Experiment 1: Effect of dietary protein. To study the pattern of changes in histidase expression, a dietary regimen of a meal-restricted schedule was selected to synchronize the rats feeding behavior. Rats were fed an 18% casein diet for 1 d to adapt them to the powdered diet and then were randomly assigned to one of five experimental groups (24 rats/group) with free access to diets containing 0, 6, 18, 35 or 50% casein for an acclimation period of 2 d. Next, the rats were fed on a restricted schedule of 7 h (0900–1600 h) with free access to water for 10 d. On d 10, six rats per group were anesthetized with carbon dioxide and killed by decapitation at 0900, 1200, 1600 and 2100 h. This corresponded to 17 h of food deprivation, 3 and 6 h into the feeding period and after 5 h of food deprivation. Liver and skin were dissected immediately and a tissue sample was frozen in liquid nitrogen for RNA extraction; the rest was used for Western blot analysis and measurement of enzyme histidase activity.

The composition of the powdered diets is shown in Table 1. The 6% casein diet was supplemented with 0.2% L-threonine and 0.4% L-threonine to improve the nutritional quality.

Experiment 2: High histidine diet. We examined whether histidine, as substrate, stimulates Hal activity and mRNA in a manner similar to that observed in rats fed a high protein diet. Another group of rats was fed a 12% casein diet supplemented with 1.25% histidine (equivalent to the amount of histidine in a 50% casein diet); its control group was fed a 12% casein diet as described in Table 1.

Experiment 3: Glucagon treatment. To study the effect of glucagon on liver histidase, rats with free access to a 10% casein diet (Table 1) were injected intraperitoneally with glucagon [0.6 mg/(100 g body wt · d)] as described previously (Lee and Harper 1971, Lee et al. 1972, Morris et al. 1973, Pestañá 1969) divided in three equally spaced doses over a 4-d period. On the last day, groups of three rats were killed 3, 6 and 12 h after the last dose to find the optimal response. Livers were immediately removed and used as above for Northern blot analysis and measurement of Hal activity. Control rats were fed a 10% casein diet and were injected intraperitoneally with saline.

Experiment 4: Half-life estimation. The apparent half-life of histidase activity was estimated by feeding rats an 80% casein diet (Table 1) for 7 d followed by a protein-free diet for 5 d according to Lee et al. (1972) and Schirmer and Harper (1970). To estimate the rate of disappearance of Hal-mRNA, rats were killed at short intervals as described by Ogawa et al. (1991). Livers were removed at 0, 6, 12, 24, 36, 48, 72 and 96 h after initiation of the protein-free diet. The half-lives of the enzyme and Hal-mRNA were estimated according to the method described by Schirmer and Harper (1970).

Histidase activity and kinetic constants. Liver or skin (1 g) were washed with ice-cold saline, minced in 4 mL of an ice-cold solution containing 5 mmol/L NaOH in 14 mmol/L KCl with a polytron (PT2000 Kinematica, Lucerne, Switzerland) at the lowest setting. The homogenates were centrifuged for 60 min at 105,000 × g, and the clear supernatant was stored at −80°C before histidase activity was measured. The assay was assayed as described (Spalter and Baldridge 1963). The method is based on the spectrophotometric measurement of the appearance of urocanic acid at 277 nm. The reaction was linear for 10 min at 25°C in 0.1 mol/L pyrophosphate buffer, pH 9.2. An enzyme unit was defined as the formation of 1 nmol of urocanic acid/min.

The protein concentration was measured by biuret assay with bovine serum albumin standards. The apparent kinetic constants of Hal were determined in high speed liver supernatants of rats fed a 6, 18 or 50% casein diet. Values for $K_m$ and $V_{max}$ were determined using the kinetic package in the DU640 spectrophotometer (Beckman, Palo Alto, CA).

Purification of histidase and preparation of antibodies. Histidase was purified from adult female rat liver according to the protocol of Berger and Harper (1976) by DEAE-Sephadex and QAE-Sephadex chromatographies. On SDS PAGE gel electrophoresis, the final protein fraction exhibited a single band of 72.2 kDa with biological activity and was purified ~50-fold. Purified homogenous histidase (200 μg) was emulsified in an equal volume of complete Freund’s adjuvant. This suspension was injected subcutaneously into a rabbit and was followed by a second injection 2 wk after the first with the same concentration of protein mixed with incomplete Freund’s adjuvant; one booster injection was applied 2 wk later. Blood was collected 2 wk after the last immunization (Lane 1988). The immunoglobulin fraction of the antisera was partially purified by the procedure of Ausubel et al. (1995); this fraction recognized a single band of 72 kDa in Western blot and neutralized more than 90% of histidase activity in liver cytosolic samples.

Immunoblot analysis. To establish if the increase in Hal activity in response to dietary protein was proportional to the amount of Hal protein in the liver cytosolic fraction, the rats were fed an 18% casein diet on consecutive d of the experiment. The liver cytosolic fraction was prepared by DEAE-Sephadex and QAE-Sephadex chromatographies. For Northern blot analysis, total RNA was isolated from liver and skin according to Chomczynski and Sacchi (1987). For Northern analysis, 20 μg of RNA was electrophoresed in a 0.8% agarose gel containing 37% formaldehyde, transferred to a nylon membrane filter (Hybond-N+) and cross-linked with a UV crosslinker (Amersham). The cDNA probe was a 1.95-kb polymerase chain reaction (PCR) product amplified from rat liver histidase cDNA kindly provided by R.R McInnes (The Hospital for Sick Children, Toronto, Canada).

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citrate)/0.1% SDS at room temperature for 20 min and then twice for 15 min with 0.1x SSC/0.1% SDS at 65°C. Digitized images and quantification of radioactivity (cpm) of the bands were done by using the Instant Imager (Packard Instrument, Meriden, CT). Membranes were also exposed to Extascan film (Kodak, Mexico) at -70°C with an intensifying screen.

Statistics. Values are means ± SEM. Analysis of significant differences among groups and over time was by two-way ANOVA followed by Scheffe’s test (Statview Statistical Analysis Program, V.4.5, Abacus Concepts, Berkeley, CA). The effect of histidine was analyzed by Student’s t test and the glucagon effect by one-way ANOVA followed by Scheffe’s test. Differences with a P-value ≤0.05 were considered significant.

RESULTS

Effect of diet on weight gain. Rats from all groups gained weight when they were allowed free access to the experimental diets (Fig. 1). By d 1, after introduction of the 7-h meal feeding schedule, all of the rats lost weight. By d 2, rats fed an 18, 35 or 50% casein diet began to gain weight, whereas rats fed a 6% casein diet did not gain weight until 3 d after the beginning of the restricted meal feeding schedule; rats fed a 0% casein diet lost weight. By d 3 and 10, weight gain was 5.6 ± 0.3, 5.9 ± 0.3 and 5.2 ± 0.3 g/d in the groups fed an 18, 35 or 50% casein diet, respectively, and rates did not differ significantly. In the protein-restricted group (6% casein diet), the weight gain was 1.1 ± 0.06 g/d. Rats fed a protein-free diet (0% casein diet) lost weight continuously (−1.4 ± 0.08 g/d) throughout the 10-d period.

Diurnal variation of histidase. Hal activity was the highest at the end of the food deprivation period (0900 h) in the groups of rats fed a 0, 6 or 18% casein diet (Table 2). Feeding resulted in a significant decrease in measurable Hal activity after 3 h in the same groups. After 6 h of feeding or 5 h of food deprivation in rats fed 0, 6, or 18% casein, Hal activity was significantly lower than in rats deprived of food for 17 h, excluding rats fed an 18% casein diet for 6 h. Rats fed a 35 or 50% casein diet did not show significant differences in Hal activity throughout the day. Northern blot analysis revealed a similar pattern (data not shown).

Measurement of kinetic constants. The results in Figure 2 show a hyperbolic response with an apparent $K_{m}$ of 5.9 ± 1.9, 5.9 ± 1.1 and 6.6 ± 1.4 mmol/L for the rats fed 6, 18 and 50% casein, respectively, which were not significantly different. $V_{max}$ values were 0.43 ± 0.04, 0.84 ± 0.05 and 2.5 ± 0.2 nmol urocanic acid/(min · mg protein) for the groups fed 6, 18 and 50% casein, respectively. Apparent $V_{max}$ values were 0.95- and 4.7-fold higher ($P < 0.01$) in the 18 and 50% protein groups than in the protein-restricted group.

Effect of dietary protein on Hal activity. After 6 h of feeding, liver Hal activity increased significantly with increasing dietary protein ($r = 0.986, P < 0.01$). However, there was a biphasic response; when protein intake was between 0 and 18%, the increment in Hal activity was 0.49 ± 0.04 nmol/(min · mg protein), whereas in rats fed between 18 and 50%, the increment was 1.87 ± 0.2 nmol/(min · mg protein). Hal activity in rats fed a 50% casein diet was sevenfold higher than in rats fed a 0% casein diet. After rats had been fed for 6 h, skin Hal was not affected by dietary protein, and the mean skin Hal activity was 78% lower than hepatic Hal of rats fed an 18% casein diet (Fig. 3A).

### TABLE 1

**Composition of diets containing different concentrations of protein**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0% casein</th>
<th>6% casein</th>
<th>10% casein</th>
<th>12% casein</th>
<th>18% casein</th>
<th>35% casein</th>
<th>50% casein</th>
<th>80% casein</th>
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<td>270.0</td>
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<tr>
<td>Mineral mixture2</td>
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<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
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</table>

1 Teklad test diets, Madison, WI.
2 Rogers-Harper, Teklad test diets, Madison, WI (Rogers and Harper 1965).
3 Vitamin mix, Teklad 40060 (mg/kg diet): p-aminobenzoic acid, 110; ascorbic acid, 991; biotin, 0.4; vitamin B-12, 30; calcium pantothenate 66; choline dihydrogen citrate, 3497; folic acid, 2; inositol 110; menadione, 50; niacin, 99; pyridoxine HCl, 22; riboflavin, 22; thiamin HCl, 22; retinyl palmitate, 40; cholecalciferol, 4; vitamin E acetate, 242.
4 Sigma Chemical, St. Louis, MO.

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**FIGURE 1** Weight gain of rats fed different concentrations of dietary protein. Values are means ± SEM, n = 24. Different letters indicate significantly different growth rates, $P < 0.05$. During the pretreatment period, rats consumed the experimental diets ad libitum. In the treatment period, each group was fed the same diet on a restricted schedule of 7 h.
HISTIDASE EXPRESSION AND DIETARY PROTEIN

TABLE 2

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Dietary casein (%)</th>
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<tbody>
<tr>
<td></td>
<td>Histidase activity, nmol urocanic acid/(min · mg protein)</td>
</tr>
<tr>
<td>0900 h (17-h food deprivation)</td>
<td>0.53 ± 0.02a,3</td>
</tr>
<tr>
<td>1200 h (3-h feeding)</td>
<td>0.34 ± 0.01b,3</td>
</tr>
<tr>
<td>1500 h (6-h feeding)</td>
<td>0.34 ± 0.01b,4</td>
</tr>
<tr>
<td>2100 h (5-h food deprivation)</td>
<td>0.26 ± 0.02c,4</td>
</tr>
</tbody>
</table>

1 Results are expressed as means ± SEM, n = 6.
2 Values in a column with different letter superscripts and those in a row with different numbers are significantly different (P < 0.05). Statistical analysis was done by two-way ANOVA with % casein and time of day as independent variables. When a significant interaction was found, differences between groups were determined by Scheffé’s test.

Effect of dietary protein on Hal concentration. The amount of histidase was eightfold greater in rats fed a 50% casein diet than in those fed a 6% casein diet (Fig. 3B). However, the increment in the amount of Hal was not proportional to the concentration of protein in the diet. Supernatants of rats fed a 0% casein diet had very low concentrations of Hal. The concentration of histidase in rats fed an 18% casein diet was 1.2-fold higher than in rats fed a 6% casein diet; Hal concentration was 2.4-fold greater in livers of rats fed 35% casein compared with the group fed 18% casein, and the increase in Hal concentration was only 20% in the group of rats fed a 50% casein diet compared with the group fed a 35% casein diet. A significant correlation (r = 0.99) between Hal activity and Hal concentration was found.

Effect of dietary protein on histidase mRNA concentrations (Hal-mRNA). As shown in Figure 3C, the Hal-mRNA concentrations increased with increasing dietary protein. The concentrations of Hal-mRNA were 1.0-, 2.8-, 7.2- and 7.5-fold higher for the groups fed 6, 18, 35 or 50% casein than for the group fed a 0% casein diet. The concentration of the Hal-mRNA measured by electronic autoradiography correlated significantly with Hal activity (r = 0.97). There was no effect of dietary protein on Hal-mRNA expression in skin (not shown).

Disappearance of histidase activity and mRNA. Figure 4 shows the decay of Hal activity and mRNA after a change from an 80% casein diet to a protein-free diet. On d 1 after the diet change, the mRNA had decreased dramatically, and barely detectable mRNA remained on d 4. A semilogarithmic plot of the concentrations of Hal-mRNA expressed as radioactivity from d 0 to 4 vs. time yielded a half-life of 17 ± 1.6 h. In contrast, Hal activity was relatively constant for 1 d and then decreased slowly over the next 3 d, resulting in a half-life of 2.8 ± 0.28 d.

Effect of a high histidine diet on histidase activity and mRNA concentrations. Rats fed a 12% casein diet supplemented with 1.25% histidine had histidase activities and mRNA concentrations that did not differ from those of rats fed a 12% casein diet, indicating that histidine did not modify Hal activity and expression of Hal-mRNA (Table 3 and Fig. 5).

Effect of glucagon on histidase activity and mRNA concentration. Within 3, 6 and 12 h of the last glucagon injection, Hal activity was stimulated 2.5-, 3.0- and 2.3-fold, respectively, compared with the control group, and there were no significant differences among them (Table 3). The increment in Hal activity 6 h after the last glucagon injection was accompanied by a fivefold increment in Hal-mRNA concentration (Fig. 5).

DISCUSSION

Histidase is one of the liver amino acid-degrading enzymes that is controlled by dietary protein. When an animal has consumed a low protein diet, the amino acids are preferentially channeled to protein synthesis as an important mechanism for regulation of amino acid concentrations in body fluids, rather than in reactions that shunt them into degradative pathways. After the ingestion of a high protein diet, the excess of amino acids must be degraded to prevent amino acids from accumulating in the body in amounts that might be toxic to the animal because of the lack of a reservoir in which to store amino acids.

Amino acid-degrading enzymes, including histidase, function at their maximum rate when amino acid concentrations...
Requirement for histidine in rats did not induce Hal activity or mRNA, indicating that the nutritional regulation of histidase gene expression is by the dietary protein and not by its substrate. Previous studies in rats (Kang-Lee and Harper 1977) have shown that the ingestion of diets supplemented with histidine above its requirement increased histidase oxidation without changing histidase activity. The results in the present and previous studies illustrate that there are two possible mechanisms for regulating histidine catabolism: 1) low protein/high histidine diets increase the rate of histidine oxidation without changes in histidase activity and expression because the high K_m of histidase ensures the oxidation of histidine over a wide range of histidine concentrations in the liver; and 2) high protein diets induce histidase expression, as observed in this study, by increasing the amounts of histidase and its mRNA. The latter mechanism might be affected by hormonal changes.

After the ingestion of a high protein diet (Hoffer 1994, Munro 1970) or administration of an amino acid mixture (Ohneda et al. 1968), plasma amino acid concentrations increase and stimulate the secretion of glucagon by the pancreas. Recently it has been proposed that α-cells of the pancreas regulate glucagon in part by an amino acid–dependent mechanism (Shay et al. 1994). It has also been demonstrated that the injection of glucagon or dibutyryl cyclic AMP stimulates histidase activity, as measured in liver and skin high speed supernatants of rats fed a 0, 6, 18, 35 or 50% casein diet.

TABLE 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hal activity (nmol/min·mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High histidine diet</td>
<td></td>
</tr>
<tr>
<td>12% casein</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>12% casein + 1.25% histidine</td>
<td>0.50 ± 0.05</td>
</tr>
<tr>
<td>Effect of glucagon</td>
<td></td>
</tr>
<tr>
<td>10% casein</td>
<td>0.44 ± 0.05b</td>
</tr>
<tr>
<td>10% casein + glucagon (3 h)</td>
<td>1.56 ± 0.13a</td>
</tr>
<tr>
<td>10% casein + glucagon (6 h)</td>
<td>1.76 ± 0.15a</td>
</tr>
<tr>
<td>10% casein + glucagon (12 h)</td>
<td>1.47 ± 0.13a</td>
</tr>
</tbody>
</table>

1 Rats fed a high histidine diet were fed a 12% casein diet supplemented with 1.25% histidine or fed 12% casein diet (controls). Rats injected with glucagon (3, 6 or 12 h after the last injection) were fed a 10% casein diet or injected with saline and fed a 10% casein diet (controls).

2 Values are means ± SEM, n = 4–6 rats. Values with different letter superscripts are significantly different (P < 0.05).
Hal activity (Lamartiniere and Feigelson 1977, Lee and Harper 1971). In this study, glucagon not only increased Hal activity, but also produced an increase in Hal-mRNA, indicating that this hormone is probably a regulator of the transcription of the Hal gene. Recently, the promoter sequence of the human Hal gene was reported; it has several CAMP responsive cis-acting elements, which include consensus sequences for transcriptional factors AP-1 (activator protein-1) and ATF (activating transcription factor) (Suchi et al. 1995). Even though the cloning of the rat histidase gene has not yet been accomplished, the homology of human histidase cDNA with rat histidase is 87% (Suchi et al. 1993), suggesting that the regulatory elements in the promoter do not differ considerably. Further studies are necessary to establish the complete set of transcriptional factors that modulate the rate of transcription of the Hal gene.

Interestingly, the nutritional regulation of histidase by dietary protein was seen only in liver. Epidermal histidase activity was 80% lower than liver histidase, and no changes in the activity and expression of epidermal histidase occurred in rats fed different concentrations of dietary protein, indicating that the transcriptional control of the Hal gene is liver specific. We have demonstrated that Hal activity and Hal-mRNA are absent in muscle, kidney, heart, brain, intestine, stomach, lung and lactating mammary tissue (N. Torres, L. Martínez, G. Alemán, H. Bourges and A. R. Tovar, unpublished results). The difference in the pattern of Hal expression in liver and skin can be explained by the differences in the catabolism of histidine in both tissues. In the liver, Hal is the first enzyme in the several steps of histidine degradation. However, the metabolism of histidine in the epidermis is different. Urocanase, the second enzyme in the metabolism of histidine, is not naturally present in the skin, and consequently, urocanic acid accumulates in the epidermis. Urocanic acid protects DNA from photomutagenesis, because the absorption spectra of both cis and trans isomers overlap with the absorption spectrum of DNA (Taylor et al. 1991), a function that remains constant and is not affected by diet.

The effect of dietary protein on Hal concentration and mRNA levels in the liver depends on both the rate of synthesis and the rate of degradation, but the time required to approach a new steady state is determined by the rate of degradation. The apparent half-life of hepatic histidase was 2.8 d, comparable to that reported previously (Lee et al. 1972, Schirmer and Harper 1970). The half-life of Hal-mRNA estimated in this work was 17.7 h, 74% shorter than the half-life of the enzyme. This may explain why during the first day after the diet change, Hal activity determined at 0900, 1500 and 2100 h was relatively constant, whereas Hal-mRNA concentration decayed rapidly. Thus, Hal activity did not show diurnal variation independently of the dietary protein concentration (Table 1). However, rats that were food deprived for 17 h showed Hal activities higher than after refeeding, possibly because during starvation there is a gradual fall in serum insulin accompanied by a rise in serum glucagon and glucocorticoids, increasing the activity of gluconeogenic enzymes.

The results of this work clearly establish that Hal expression is controlled by the dietary protein concentration and presumably is partially regulated by glucagon. Additional studies are required to establish the nature of the transcriptional control of the Hal gene. Such studies are currently underway in our laboratory. In the future, the establishment of the common regulators of the expression of the amino acid-degrading enzymes will give information at the molecular level concerning the basic mechanisms that control body nitrogen.

**LITERATURE CITED**


