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Analysis of the Rates of Dietary Induced Changes in Chick Kidney Arginase Activity (40875)[1]

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Abstract. An investigation was carried out to determine the turnover rate of dietary induced changes in chick kidney arginase. The enzyme activity in kidney was increased or decreased following addition or removal of certain dietary amino acids to the diet of chicks. Excesses of dietary arginine or lysine caused the kidney arginase to increase to a new steady-state level with a half-time of 2.6 to 3.8 days, whereas when these amino acids were removed from the diet or when AIB was added to the diet, the activity rapidly declined to a much lower level with a shorter half-time of 0.8–1.9 days. The rate constant for degradation was changed from 0.18–0.27 to 0.36–0.90 under these conditions. The results suggest that these dietary amino acids might alter the rate of arginase degradation in chick kidney.

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) activity in chicken kidney can be altered by a number of dietary modifications that increase or decrease enzyme activity in kidney tissue. Excesses of dietary lysine (1–3) seem particularly effective in causing elevation of chicken kidney arginase activity but several other amino acids when added in excess to diets for chicks, such as arginine, ornithine, histidine, isoleucine, and tyrosine also cause increases in arginase activity (2, 3). The amino acids, glycine, threonine, and α-aminoisobytric acid (AIB), on the other hand, markedly depress arginase activity in chicken kidney (3, 4).

These changes in enzyme activity seem to be due to alterations in enzyme protein levels in kidney tissue since partial purification of the enzyme resulted in isolation of activity in proportion to the activity seen in the original homogenate (5). However, complete purification of the enzyme has not been accomplished.

Although several studies have been published describing dietary effects on kidney arginase activity in chicken kidney (2, 3, 6), a detailed study of the rates at which these changes are induced has not been published. This paper describes rates of change that take place in the activity of chick kidney arginase under several dietary conditions.

Schimke (7) has extensively reviewed a number of techniques to estimate the rate of turnover of various enzymes on the basis of time course of changes in enzyme level. These techniques assume that enzyme activities decay exponentially to basal levels after removal of some agent that has increased the level significantly over basal levels. Thus, the rate of enzyme degradation can be expressed in terms of a first-order rate constant, while enzyme synthesis generally has conformed to zero-order kinetics. The model for such a change in tissue content of an enzyme is: $dE/dt = S - kE$. Where E is the content of enzyme, S the rate constant for synthesis, and k the first-order rate constant for enzyme degradation. The rate of change can be also estimated from time course of approach of an enzyme to a new steady state. The equations derived from the same model indicate that the time course whereby an enzyme approaches a new steady state is only determined by the rate constant of degradation, and the time taken to increase to one-half of the final increase at the steady state is equal to the half-life of the enzyme. In this study, we have used both the decay and approach to steady-state techniques for the analysis of arginase turnover.

Materials and methods. The chicks used

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in the first three experiments were from a commercial white leghorn strain. A strain of chicks selected for a high requirement (HA) for arginine (8) was used in the last experiment.

Feeding experiments were carried out in thermostatically controlled electrically heated battery brooders with raised wire floors. Chicks were fed various types of diets and water *ad libitum*. The diets were all semipurified diets containing casein or soybean protein as protein sources which were described by Nesheim (2) or containing corn gluten meal as reported by Wang *et al.* (6). Supplements of amino acids were added to the diets at the expense of glucose. Arginase activity was determined in kidney homogenates under the condition described by Tamir and Ratner (9). Urea was measured by the automated method of Marsh *et al.* (10).

Analysis of the enzyme turnover in tissue and the calculation of degradation rate constants were according to the methods described by Schimke (7). The changes in enzyme activity were determined over the period of time required for the activity to reach a new steady state following dietary change. It is assumed that the change observed was due to the alteration in enzyme concentration. The rate constant for degradation (K_d) was estimated by the slope of the straight line obtained from a semilogarithmic plot of $(E_t) - (E_{ss,0})$, or $(E_{ss'}) - (E_t)$ versus time, where

E_t = enzyme activity at any time, t;
$E_{ss,0}$ = enzyme activity of the lower steady state;
and
$E_{ss'}$ = enzyme activity of the higher steady state.

The half-life ($t_{1/2}$) was calculated from the equation, $t_{1/2} = 0.693/K_d$.

Results. In the first experiment groups of chicks were changed from a commercial chick starter[3] to a diet containing casein to which 1.5% L-arginine–HCl was added. Chicks were sacrificed every 2 days for 12 days and arginase activity was determined in kidney homogenates. After 2 weeks, the remaining chicks were again fed the commercial chick starter and enzyme determinations in chick kidneys were made daily for 5 days and then again after 8 days. The results are shown in Fig. 1, A and B. Changing from the chick starter to the casein diet containing 1.5% arginine–HCl resulted in a rise in enzyme activity of approximately twofold. The half time of the change from one steady state to another was about 3.8 days. When chicks were changed from feeding on the casein diet, containing 1.5% L-arginine–HCl to the chick starter diet, the enzyme activity in the kidney fell rapidly to the original preexperimental level with a half-time of 1.5 days. The rate constant for enzyme degradation was doubled when the chicks were returned to the chick starter diet.

In the second experiment (Fig. 1, C and D) chicks previously fed a purified diet containing soybean protein for 2 weeks were fed the same diet supplemented with 1% AIB. Kidney arginase activity was measured daily for the first 4 days and then every 2 days up to 2 weeks of feeding the diet with AIB. The arginase level in kidney declined rapidly to about one-sixth of that found in chicks before the diet with AIB was fed. The half-time of the change was only 0.8 days and the degradation rate constant was 0.90. When ornithine was added to the basal diet, in addition to the AIB (Fig. 1D) very little rise in enzyme activity occurred, even though in the absence of AIB ornithine normally raises the level of arginase activity in chicken kidney (2).

In the third experiment (Fig. 1, E and F), chicks were changed (Fig. 1E) from a commercial chick starter diet to the basal diet containing soybean protein that was supplemented with 2% L-arginine–HCl and kidney arginase was measured at intervals following the dietary change. The arginase activity rose about threefold over the feeding period with a half-time of 2.6 days for the change of enzyme level to the new steady state. The degradation rate constant was 0.27. When the diet was changed to the soybean basal plus 1% AIB (Fig. 1F) the enzyme level in kidney rapidly fell to less than one-tenth that at Day 0. This decline

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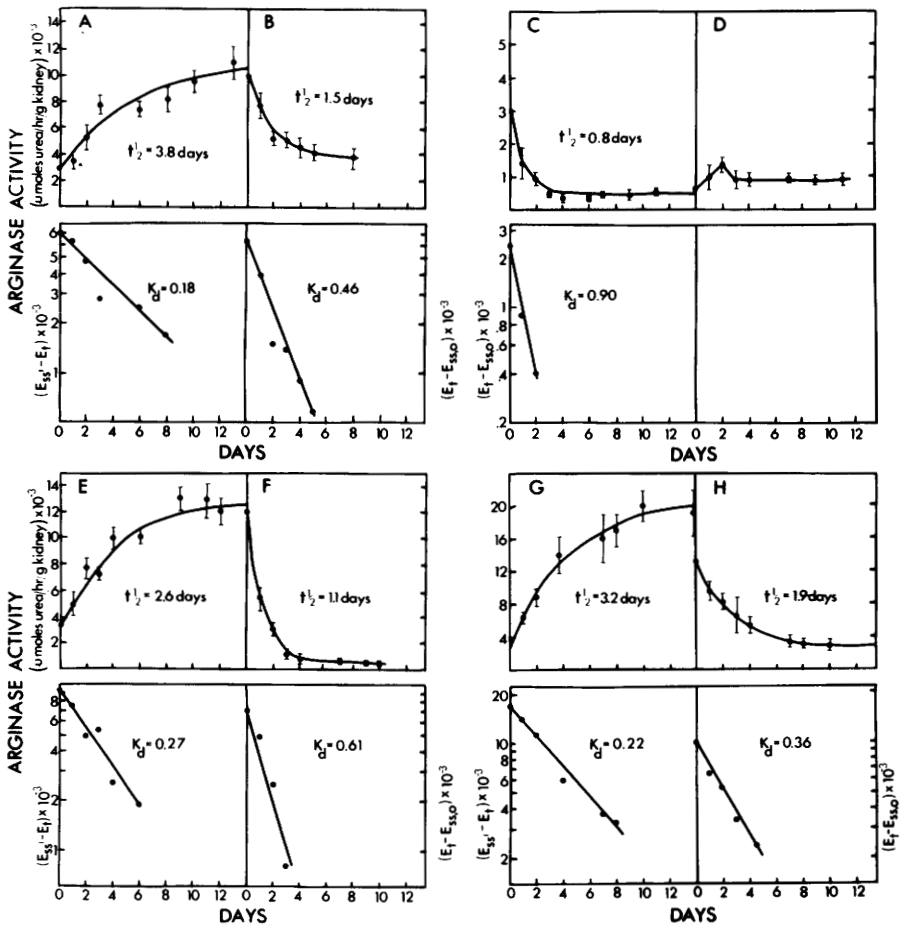

FIG. 1. Determination of the rates of change in kidney arginase activity when chicks receive various dietary treatments. The initial and the final dietary treatments in each experiment are as follows: See the text for diet descriptions. (A) Chick starter → casein basal + 1.5% L-arginine–HCl; (B) casein basal + 1.5% L-arginine–HCl → chick starter; (C) chick starter → soybean basal + 1% AIB; (D) soybean basal + 1% AIB → soybean basal + 1% AIB + 1% L-ornithine–HCl; (E) chick starter → soybean basal + 2% L-arginine–HCl; (F) soybean basal + 2% L-arginine–HCl → soybean basal + 1% AIB; (G) corn gluten basal → corn gluten basal + 1% L-lysine–HCl; and (H) corn gluten basal + 1% L-lysine–HCl → corn gluten basal. Chicks from HA strain (7) were used in experiments G and H. The diets were changed on Day 0 after chicks were fed the initial diets for 2 to 3 weeks. Each point represents mean ± SE for duplicate determinations on kidney homogenates from four to five individual chicks. K_d is the first-order rate constant for enzyme degradation. $t_{1/2} = 0.693/K_d$. E_t = enzyme activity at any time. $E_{ss,0}$ = enzyme activity of the lower steady state. $E_{ss'}$ = enzyme activity of the higher steady state.

had a half-time of 1.1 days and a degradation rate constant of 0.61.

In the final study (Fig. 1, G and H), the chicks of HA strain were changed from the basal diet containing corn gluten meal to the same diet supplemented with 1% L-lysine–HCl. The arginase levels (Fig. 1G) determined in chicks sampled at intervals rose about fourfold over the experimental period with a half-time of 3.2 days and a degradation rate constant of 0.22. When the chicks were returned to the basal diet, without added lysine, the arginase level fell (Fig. 1H) rapidly with a half-life of 1.9 days.

Similarly the degradation rate constant increased to 0.36 under these conditions.

Discussion. These experiments demonstrate the remarkable effect that certain dietary amino acids have on the level of arginase activity found in chicken kidney. They also show that the rates of change of enzyme activity vary depending on the stimulus responsible for the change. When enzyme level was increased the half-time required to reach the new, higher level of enzyme activity was about 2.6 to 3.8 days whereas when the stimulus was removed, or when AIB was added, the arginase level changed rapidly to a lower level with a half-life from 0.8 to 1.9 days. This was accompanied by marked increase in the degradation rate constant, suggesting that the stimulation of arginase activity appeared to be, in part, due to a decrease in the rate of arginase degradation. Although the present experiments do not provide actual measurements of the rates of enzyme synthesis and degradation of the arginase molecule they do suggest that such changes must be taking place, particularly in rates of enzyme degradation.

There is some evidence that enzymes may be stabilized by substrate or cofactors present in the tissue. Schimke *et al.* (11) showed that tryptophan reduced the rate of tryptophan oxygenase degradation *in vivo*. Katunuma *et al.* (12–14) reported the presence of various group-specific proteases which initiate the degradation of several pyridoxal-, NAD-, or FAD-dependent enzymes. Apoenzymes were susceptible while holoenzymes were resistant to these proteases.

The mechanism involved in arginase degradation in the tissue is unknown. However, it is interesting that amino acids that cause increased enzyme activity in avian kidney are also amino acids that are either substrate, or strong competitive inhibitors of the enzyme. Kadowaki and Nesheim (5) showed that ornithine and lysine were strong competitive inhibitors of the enzyme (K_i = 2–5 mM). Our unpublished data show that isoleucine is also a strong competitive inhibitor (K_i = 2 mM) of chick kidney arginase. AIB on the other hand is a rather weak competitive inhibitor (K_i = 50–70 mM) of the enzyme (5). Conceivably, high levels of the amino acids that interact strongly with the enzyme may stablize the enzyme and reduce the rate of degradation causing the enzyme activity in tissue to be changed to a higher steady-state level. More detailed studies of the purified enzyme will be needed to clarify the mechanism whereby the various amino acids can affect arginase activity in chicken kidney.

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