# Amino Acid Transport by Rat Parathyroid Glands *in vivo*: Effect of Low Calcium Diet. (29332)

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Serum ionized calcium concentration is considered to be the major, if not the sole, physiologic regulator of parathyroid hormone synthesis and/or release(1,2), though the mechanism by which this is accomplished is unknown. During sustained dietary calcium deprivation, with resultant moderate reduction of serum calcium concentration, parathyroid gland activity is apparently increased, as indicated by increase in size of the parathyroid gland and prevention of a marked fall in serum calcium level(3). However, there is not yet available a parathyroid hormone assay with sufficient sensitivity and accuracy to measure the quantity of parathyroid hormone secreted under normal physiologic conditions, nor the magnitude of increase in secretion of this polypeptide hormone in response to physiologic or pathologic stimuli.

The active transport of amino acids into cells is an early critical step in protein synthesis, and the rate and magnitude of this transport can be used as a measure of the protein synthesis(4,5,6). The non-metabolizable amino acid $\alpha$-aminoisobutyric acid (AIB) is transported into cells similarly to naturally occurring amino acids, but is neither degraded nor incorporated into protein(5). Therefore, by using this amino acid, it has been possible to isolate and study the transport process in several tissues, and to evaluate the effect of various hormonal and other stimuli on this process(4-8).

In the present study, the *in vivo* active transport of AIB into parathyroid and thyroid cells of rats with normal calcium intake, or with low calcium intake, was studied to determine: a) the relative amino acid transport into cells of these 2 tissues under control conditions; b) the relative amino acid transport by these 2 tissues with low calcium stimulus, and c) the magnitude of change in amino acid transport into parathyroid cells in response to sustained low calcium stimulus.

*Materials and methods.* Female Sprague-Dawley rats of the same age (8 weeks) and initial weight (140-160 g) were divided into 2 groups. The control group received a normal stock diet containing 1.7% calcium and 0.8% phosphorus. The other group of rats received a diet which was similar except that it contained no discernible calcium and 2½% phosphorus. After 21 days, the average weight of the control group was 200 g and of the low calcium group 180 g. Each rat then received 15 $\mu$c $\alpha$-aminoisobutyric acid-1-$C^{14}$ (AIB-1-$C^{14}$) (specific activity, 9.96 mc/mmole) intraperitoneally 20 hours before sacrifice and 7 $\mu$c $NaCl^{36}$ (specific activity, 0.357 mc/g) intraperitoneally 2 hours before sacrifice. The rats were allowed food until killed by exsanguination. The parathyroid glands and small specimens of thyroid gland were removed under dissecting microscope observation. The tissue specimens were weighed in sealed containers on a microbalance, dried to constant weight, reweighed, placed in a counting vial and dissolved in Hyamine hydroxide. Scintillator solution (Toluene-PPO-POPOP) was added and the $C^{14}$ and $Cl^{36}$ activity determined with a liquid scintillation counter (Packard Tri-Carb, model 314 EX). Counting efficiency was determined by addition of internal standards (toluene-$C^{14}$ and 1,2-dichloroethane-$Cl^{36}$) to each counting vial. Sufficient counts were accumulated to reduce the counting error to less than 2%. Total tissue water was calculated as wet weight minus dry weight for each tissue sample. Extracellular water (ECW) volume was obtained by determining chloride space by isotope ($Cl^{36}$) dilution. Intracellular water (ICW) volume was calculated as the difference between total tissue water and extracellular water. These fluid compartments

TABLE I. Effect of Dietary Calcium Intake on Parameters of Parathyroid Function. All values are expressed as the mean ± standard error.

| Diet | No. rats | Serum Ca (mg %) | Serum P (mg %) | Parathyroid glands wt (mg) | Distribution ratio [AIB (ICW/ECW)] |
|---|---|---|---|---|---|
| Control | 7 | 10.2 ± .1 | 7.6 ± .3 | .295 ± .087 | 5.9 ± 1.0 |
| Low Ca, high P | 7 | 8.6 ± .2 | 10.6 ±1.4 | .636 ± .126 | 17.5 ± 4.1 |

were determined on each tissue specimen which was analyzed for AIB-1-$C^{14}$. The results were expressed as the distribution ratio of AIB, *i.e.*, concentration of AIB-1-$C^{14}$ in ICW to its concentration in ECW [AIB(ICW/ECW)]. Serum obtained at time of sacrifice was analyzed for $C^{14}$ and $Cl^{36}$ as above, and for calcium(9) and phosphorus(10).

*Results.* As shown in Fig. 1, the active transport of AIB-1-$C^{14}$ by parathyroid cells was much greater ($P<0.001$) than that by the thyroid cells under normal dietary conditions. As shown in Table I, the low calcium, high phosphate diet for 3 weeks resulted in a moderate but significant decrease ($P<0.001$) in serum calcium, a slight increase ($P<0.05$) in serum phosphorus, and a greater than doubling (2.2 ×) in parathyroid weight ($P<0.001$). There was a tripling (3.0 ×) in active transport of AIB-1-$C^{14}$ into parathyroid cells ($P<0.02$) but no change in the magnitude of transport into thyroid cells (Fig. 1).

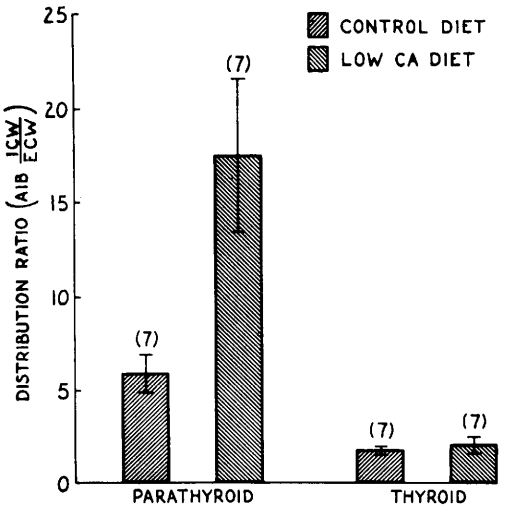


FIG. 1. *In vivo* active transport of AIB-1-$C^{14}$ into parathyroid and thyroid cells of rats maintained for 21 days on control diet or low calcium, high phosphate diet. Each bar represents mean value, with standard error, for the number of animals studied (in parentheses).

*Discussion.* The greater active transport of AIB-1-$C^{14}$ by the parathyroid than by the thyroid cells under normal conditions is unexplained. However, there is evidence that parathyroid hormone in the circulation is short-lived and the parathyroid gland must secrete hormone continuously(11). Since the biologic life of thyroid hormone is much longer than that of parathyroid hormone and the thyroid gland is adapted for abundant hormone storage, it is possible that there is less need for avid amino acid accumulation by the thyroid gland.

Though only one non-parathyroid tissue was studied, the data suggest that the increased amino acid transport as a result of low calcium stimulus is a unique response of parathyroid cells.

Multiplying the relative change in parathyroid gland weight (2.2 ×) by the relative change in active transport of AIB-1-$C^{14}$ per unit of intracellular water (3.0 ×) in response to low calcium stimulus yields a value of 6.6 times that of the control diet rats. This value may represent an approximation of the magnitude of increase in parathyroid activity in response to the stimulus used in this study.

Raisz and O'Brien(12) recently studied the effect of calcium on the active transport of AIB by the parathyroid gland in short-term *in vitro* experiments. In that study, the magnitude of increase of AIB transport in response to low calcium stimulus was less than in the present *in vivo* study, but the response was demonstrated within 2 hours.

The present study has not established whether the active transport of AIB-1-$C^{14}$ by the parathyroid cells represents accumulation of amino acids for a) hormone synthesis, b) enzyme protein synthesis, c) increased

cellular proliferation, or d) a combination of these processes. However, the observed AIB distribution ratios paralleled an accepted physiologic response (increased parathyroid gland size) to parathyroid stimulus. Therefore it is concluded that the magnitude of active transport of AIB is an additional parameter of parathyroid function which should allow determination of relative changes in parathyroid activity under various physiologic conditions.

*Summary.* Active transport of α-aminoisobutyric acid by rat parathyroid tissue was much greater than by thyroid tissue under control conditions. Calcium deprivation doubled the parathyroid weight and tripled the α-aminoisobutyric acid transport into parathyroid cells, but had no effect on thyroid cells. The magnitude of this transport phenomenon is apparently a measure of physiologic activity of the parathyroid glands.

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## Triphenyltetrazolium Reduction by Uterine Tissue of Rats.*† (29333)

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The reduction of 2-3-5 triphenyltetrazolium chloride (TTC) to formazan by the vaginal lining of the mouse has been used as a biological assay method for estrogens(1). Martin found that rate of reduction was proportional to degree of estrogenic stimulation. Progesterone, testosterone, and cortisol did not influence the reaction. Although estrogens are necessary for reproduction, excessive amounts can be harmful(2). Reproductive efficiency varies between females and within females from time to time. An index of the degree of estrogenic stimulation attained by the genitalia at time of breeding or during different stages of pregnancy should be useful in studying the reasons for some of these differences and lead to an increased understanding of the estrogen requirement for high reproductive efficiency. TTC reduction may serve as such an index.

The experiment reported here is an attempt to adapt the TTC reduction of uterine segments as an *in vitro* method of evaluating degree of estrogenic stimulation that the genitalia has attained. Changes in TTC reduction brought about in the uterus of spayed females and during different stages of the estrous cycle in intact females are reported.

*Methods.* A line-bred stock of female rats of Wistar origin 70-100 days of age was used. Immediately after killing, segments of the uterus (35-45-mg wet weight) were removed, blotted free of excess fluid and weighed to the nearest 0.1 mg. The segments were sliced

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