# Wound healing and thymotropic effects of arginine: a pituitary mechanism of action[1-4]

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ABSTRACT Supplemental dietary arginine HCl (ARG-HCl) minimizes immediate post-wounding weight loss, accelerates wound healing, and is thymotropic for uninjured and wounded rats. The present experiments were to determine if arginine-pituitary interactions underlie these effects because arginine is a growth hormone secretagogue. Effects of 1% dietary ARG-HCl supplements (0.5% added to a regular commercial rat diet containing 1.8% ARG, 0.5% in drinking water) were studied in (a) hypophysectomized (hypox) rats supplemented with ACTH, L-thyroxine, testosterone propionate, (b) such hypox rats additionally supplemented with bovine growth (hypox + bGH) hormone, (c) intact rats (Int), and (d) intact rats supplemented with growth hormone (Int, bGH). Group (a) hypox rats healed their wounds as rapidly as intact rats (dorsal skin incision breaking strength, accumulation of reparative collagen in sc polyvinyl alcohol sponges). Group (b) hypox, bGH rats showed increased wound breaking strength and accumulation of reparative collagen in the sc sponges to levels significantly greater than those of intact controls; bGH given to intact controls did not affect these indices of wound healing. Supplemental ARG-HCl given intact rats significantly minimized immediate postoperative weight loss, increased wound breaking strength and sponge reparative collagen accumulation, and increased thymic weight. None of these effects of supplemental ARG-HCl were observed in group (a) hypox rats or group (b) hypox + bGH rats. We conclude that an intact hypothalamic-pituitary axis is necessary for these beneficial effects of supplemental ARG-HCl given wounded rats. *Am J Clin Nutr* 1983; 37:786–794.

KEY WORDS Arginine, wound healing, thymus, pituitary

## Introduction

Improved nutritional care of seriously ill and injured patients, particularly the use of parenteral high calorie-amino acid mixtures, is an important medical advance. However, information regarding the optimal supply of individual amino acids for such patients is incomplete. Trauma and other stressors, including sepsis, produce significant alterations in the intra- and extracellular concentrations of certain amino acids (1, 2). The nutritional and physiological consequences of these changes are not completely understood. There is growing awareness that the dietary requirements for specific amino acids may vary in patients with injury and sepsis and that the administration of certain amino acids may modify their metabolic and physiological responses (3).

Previous studies in our laboratory have demonstrated that injured animals have an increased requirement for arginine. Thus, Seifter et al (4) have shown that arginine becomes a dietary essential amino acid for maintenance of body weight, wound healing, and survival in injured young adult rats and that a 1% dietary supplement of arginine HCl accelerates wound healing in injured rats ingesting a commercial diet containing 1.8% arginine, which supports normal growth, reproduction, and longevity of healthy rats.

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The same workers (5) demonstrated that 1% arginine HCl supplementation increases thymic weight and the number of thymic small lymphocytes in normal rats and mice and prevents or lessens the thymic involution that occurs in mice and rats subjected to various stressors, including femoral fracture. Sitren and Fisher (6) have shown that dietary supplements of arginine and glycine added together to a casein-based diet improve nitrogen balance in rats subjected to ether anesthesia or closed unilateral femoral fracture under ether anesthesia.

These findings led us to study the mechanisms by which supplemental dietary arginine ameliorates certain posttraumatic processes, such as body weight changes, wound healing, and thymic involution. We postulated that the beneficial effects of arginine are related in part to the hormone secretagogue actions of arginine (4, 5). Arginine is a well-known secretagogue of growth hormone (7), prolactin (7), insulin (8), and glucagon (9). We hypothesized that increased secretion of growth hormone in particular is a major factor in the beneficial response to arginine in injured animals. The present experiments were carried out as part of a study to test this hypothesis by investigating arginine-pituitary interrelationships in injury.

## Materials and methods

Intact male Sprague-Dawley rats and hypophysectomized (hypox) male Sprague-Dawley rats were obtained from a commercial breeder (Charles River Laboratories, Wilmington, MA). The hypophysectomies were carried out by the supplier 24 h before the animals were shipped. The hypox rats weighed 125 to 150 g at the time of pituitary ablation and intact controls were matched for sex, weight, and age. Upon arrival at our laboratory, all rats were housed individually in no 2 galvanized mesh steel cages and kept at controlled temperature (24 to 26°C) and relative humidity (35 to 40%) in a room with outside windows. All animals were allowed at least 1 wk of acclimatization to our laboratory conditions before wounding.

During the period of acclimatization, all rats ate a commercial powdered rat diet [Rockland Rat/Mouse Diet (4% fat), Teklad Inc, Monmouth, IL] ad libitum. This diet contains about 1.8% arginine and supports normal growth, reproduction, and longevity in normal intact rats. The intact rats drank tap water, while the hypox rats were given tap water (experiment 1) or 5% glucose in 0.9% saline (experiments 2 and 3) before hormonal replacement. All hypox rats that gained more than 10 g in body weight before growth hormone therapy were excluded from the experiments since continued body weight gain is an indication of incomplete pituitary ablation (10). In each experiment the rats were then divided into several groups of eight animals each according to different hormonal or dietary regimens as described below.

### *Hormonal therapy*

*Hypox rats.* ACTH (Acthar, Armour Pharmaceutical Co, Phoenix, AZ) and L-thyroxine (Synthroid, Flint Laboratories, Deerfield, IL) were dissolved in sterile bacteriostatic normal saline (0.9% NaCl) and frozen in individual aliquots. Fresh stock solutions were prepared every other day. These hormones were injected subcutaneously in a volume of 0.1 ml each. Sterile testosterone propionate (Gotham Pharmaceuticals, Brooklyn, NY) was diluted with autoclaved peanut oil to a concentration of 750 μg/0.1 ml and was injected intramuscularly. Bovine growth hormone (bGH) [this hormone was generously supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (lot #NIH-GH-B-18), Bethesda, MD] was dissolved in bacteriostatic normal saline made alkaline with a few drops of 0.01 N NaOH solution. Stock solutions were prepared fresh every 5 to 7 days and kept frozen in small, sterile vials. The quantity needed at each injection schedule was thawed 30 min before administration. The growth hormone was injected subcutaneously.

Hypox rats were started on daily administration of 0.5 IU ACTH, 1.1 μg/100 g body weight of L-thyroxine and 750 μg of testosterone propionate 2 wk after hypophysectomy in experiment 1 and 3 days after the pituitary ablation in experiments 2 and 3. Two days later, some groups of hypox rats in experiments 2 and 3 were also started on bGH injections. In experiment 2 , two groups of rats received 0.25 IU bGH/100 g body weight/once a day at 8:00 AM, while two other hypox groups received such injections three times daily at 8:00 AM, 12:00 PM, and 4:00 PM. The latter regimen was used in experiment 3. The hypox rats not receiving bGH and those receiving the single daily dose (8:00 AM) received normal saline injections at the other times. All hormonal replacement or saline injections in hypox rats were continued according to the same schedule throughout the postoperative period (**Table 1**).

*Intact rats.* Two groups of intact rats in experiment 3 were given 0.5 IU bGH/100 g body weight three times daily for 3 days pre- and 3 days postwounding. All intact groups not receiving bGH were given control injections of normal saline at comparable times.

TABLE 1
Course of hormonal therapy in hypophysectomized rats

| | Fluid before hormonal Rx | Start of ACTH, thyroxine, and testosterone Rx (days posthypox) | Start of bGH Rx (days posthypox) | Length (days) of hormonal Rx before wounding |
|---|---|---|---|---|
| Exp 1 | Tap H20 | 14 | | 14 |
| Exp 2 | D5NS | 3 | 5 | 8 |
| Exp 3 | D5NS | 3 | 5 | 8 |

* For each experiment hormonal therapy was continued throughout the postoperative period.

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*Arginine supplements*

Fourteen days (experiment 1) or 5 days (experiments 2 and 3) after the start of the ACTH, L-thyroxine, and testosterone propionate injections and 3 days before wounding, half of the intact and half of the hypox rats received a supplement of L-arginine HCl (Grand Island Biological Co, Grand Island, NY) given as a 0.5% mixture in the diet and as a 0.5% tap water solution. The arginine HCl supplementation was continued for 3 days postwounding after which the basal diet and tap water was offered; the other half of the rats ate the basal diet and drank tap water throughout the preoperative and postoperative periods. All rats ate and drank ad libitum. Animals were weighed daily pre- and postwounding; food and water intakes were measured in experiment 1.

*Wounding procedure*

Three days after they were placed on their respective diets, the rats were wounded using aseptic techniques. Intraperitoneal sodium pentobarbital (3.5 mg/100 g body weight) supplemented as needed with light ether anesthesia was used. The backs of the rats were clipped and shaved, scrubbed with organo-iodine solution (Wescodyne Scrub, West Chemical Products, Inc, New York, NY), and then a 7.0 cm paravertebral incision was made through the skin and the panniculus carnosus. At the upper poles of the wound and 5.0 cm lateral to it, subcutaneous pockets were created by inserting a pair of Metzenbaum scissors and spreading the blades. Into these pockets soft, water-moistened, sterile, porous, preweighed polyvinyl alcohol sponges were inserted. Wounds were closed with seven no 40 stainless steel sutures placed at equal distances to each other and from the wound edges. Prewounding hormonal and dietary treatments were continued postoperatively as described above. Sutures were removed on the 7th postoperative day. On the 10th postwounding day, all rats were killed by intraperitoneal injection of a lethal dose of sodium pentobarbital. The length of the skin incision was measured in situ and then the dorsal pelt, containing the incision, was excised with generous margins. The pelt was placed on the base of a multibladed guillotine, the incision adjusted to its own in vivo dimension and sectioned into seven strips, each centered by a segment of the incision and measuring 0.6 cm in width and 7.0 cm in length. Three skin strips (1, 3, and 6, number 1 being most cephalad) from each pelt were weighed and fresh breaking strength determinations were carried out immediately using a tensiometer previously described (11). The polyvinyl alcohol sponges were removed carefully, individually wrapped in aluminum foil, and kept frozen at −20°C until the hydroxyproline contents were determined by the method of Woessner (12). The thymus glands were removed, cleared of all fatty and connective tissue, and weighed on a Roller-Smith tissue balance.

All data are expressed as mean ± SEM. Unless otherwise noted, the data were analyzed by "Student's" *t*-test for small samples. Statistical significance was taken at the 95% confidence level.

## Results

Arginine supplementation did not affect food or water intake in intact and hypox rats (measured in experiment 1, **Table 2**). Preoperative or cumulative postoperative body weight gains in intact rats did not differ with arginine supplementation in any of the experiments. However, in each experiment, arginine supplementation of intact rats significantly minimized (experiment 1, −3.1 versus −9.7 g, $p < 0.02$) or obliterated the weight loss during the 1st postoperative day (1.9 versus −4.3 g, $p < 0.05$ and 4.4 versus − 3.6 g, $p < 0.05$, experiments 2 and 3, respectively) (**Table 3**, **Fig 1**).

Administration of bGH to intact rats not receiving the arginine supplementation resulted in a gain of weight during the 1st postoperative day (3.1 ± 1.3 g) while their pairs given saline rather than bGH lost weight (−3.6 ± 1.8 g); this difference was statistically significant ($p < 0.05$) (experiment 3, Table 3). Administration of bGH to arginine supplemented intact rats resulted in the largest immediate postwounding weight gain (6.9 ± 0.7 g).

The hypox rats supplemented with ACTH, thyroxine, and testosterone propionate but not bGH grew minimally (5.0 and 4.3 g during the preoperative period and 2.3 and −3.8 g postoperatively in experiments 1 and 3, respectively). Treatment of hypox rats in experiment 2 with low doses of bGH (0.25 IU/100 g body weight/once daily) resulted in an increased growth response, but not as great as the rate of growth of intact rats (22.1 versus 28.2 g for the intact rats, $p < 0.02$, preoperatively and 54.8 versus 62.4 g, p NS, postoperatively). Hypox rats given the high dose of bGH (0.25 IU/100 g body weight/tid) grew as well as intact controls (33.8 and 30.5 g versus 38.2 and 22.8 g in experiments 2 and 3, respectively, preoperatively, p NS in each

TABLE 2
Food and water intake of rats in experiment 1

| | Diet | p | Water | p |
|---|---|---|---|---|
| | *g/day* | | *ml/day* | |
| Intact | | | | |
| Basal | 32.7 ± 0.9 | NS | 57.6 ± 1.6 | NS |
| Arginine | 30.9 ± 0.7 | | 55.8 ± 1.3 | |
| Hypox* | | | | |
| Basal | 16.5 ± 0.9 | NS | 37.9 ± 2.3 | NS |
| Arginine | 16.7 ± 0.5 | | 34.8 ± 1.1 | |

* Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt), and testosterone propionate (750 μg).

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TABLE 3
Body wt (g ± SEM) data in experiments 1 and 3

| Group | Diet | 3 Days prewounding (start of diets) | Day of wounding | Body wt Δ day 12 postwounding | 10 Days postwounding (Sacrifice) |
|---|---|---|---|---|---|
| Experiment 1 | | | | | |
| Intact | Basal | 290.7 ± 2.9 | 326.4 ± 5.2 | −9.7 ± 1.7 | 369.5 ± 6.5 |
| | Arg* | 288.8 ± 5.6 | 321.9 ± 6.2 | −3.1 ± 1.9 | 364.6 ± 8.1 |
| | P | NS | NS | <0.02 | NS |
| Hypox† | Basal | 166.9 ± 1.7 | 171.9 ± 1.5 | −4.2 ± 0.9 | 174.2 ± 1.9 |
| | Arg | 170.6 ± 1.8 | 176.3 ± 2.2 | −2.1 ± 1.1 | 178.6 ± 1.5 |
| | P | NS | NS | NS | NS |
| Experiment 3 | | | | | |
| Intact | Basal | 180.8 ± 2.1 | 203.6 ± 2.6 | −3.6 ± 1.8 | 275.8 ± 2.8 |
| | Arg | 179.0 ± 1.7 | 205.6 ± 1.6 | 4.4 ± 0.9 | 274.8 ± 3.9 |
| | P | NS | NS | <0.005 | NS |
| Intact + bGH‡ | Basal | 175.3 ± 3.1 | 204.6 ± 3.7 | 3.1 ± 1.3 | 291.1 ± 6.5 |
| | Arg | 174.0 ± 2.2 | 209.4 ± 2.5 | 6.9 ± 0.7 | 298.6 ± 3.1 |
| | P | NS | NS | <0.05 | NS |
| Hypox† | Basal | 160.5 ± 4.1 | 164.8 ± 3.8 | −2.6 ± 0.3 | 161.0 ± 3.6 |
| | Arg | 157.6 ± 4.0 | 163.4 ± 4.1 | −2.8 ± 0.5 | 163.8 ± 2.6 |
| | P | NS | NS | NS | NS |
| Hypox† + bGH§ | Basal | 176.5 ± 2.5 | 207.0 ± 3.1 | 1.8 ± 0.7 | 271.8 ± 3.6 |
| | Arg | 178.5 ± 4.7 | 215.6 ± 5.2 | 3.1 ± 0.6 | 282.9 ± 6.5 |
| | P | NS | NS | NS | NS |

* 0.5% supplement in diet and 0.5% solution for drinking.
† Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt) and testosterone propionate (750 μg) as noted in text.
‡ Daily bGH (0.5 IU/100 g body wt tid) as noted in text.
§ Daily bGH (0.25 IU/100 g body wt tid) as noted in text.

case, and 57.2 and 64.8 g versus 62.4 and 72.2 g postoperatively, respectively, p NS in each case).

Arginine supplementation did not affect weight gains or losses in any of the hypox groups, regardless of their hormonal therapy.

### *Wound healing*

No animal developed wound infection and there were no differences in the gross appearances of the wounds.

Wound skin strips of arginine HCl-supplemented intact animals weighed significantly more than those of the unsupplemented intact controls (**Tables 4, 5, and 6**). Arginine supplementation also significantly increased wound breaking strength of both saline-injected (Tables 4, 5, and 6) and bGH-injected rats (Table 6). The increases in wound breaking strengths in the intact rats were paralleled by statistically significant increases in reparative collagen accumulation in the subcutaneously implanted polyvinyl alcohol sponges as assessed by their hydroxyproline contents. In contrast, bGH treatment of intact rats fed the basal or arginine supplemented diet with bGH did not increase wound healing when compared to their respective saline-injected intact controls (Table 6).

Hypox rats had very thin pelts and their skin strips weighed about 50% less than those of intact rats ($p < 0.001$) (Tables 5 and 6). Hypox rats fed the basal diet and treated with ACTH, L-thyroxine, and testosterone propionate had wound breaking strengths equal to those of intact animals in experiment 2 (240 ± 13 versus 218 ± 16 g, p NS) (Table 5), but somewhat less than intacts in experiments 1 and 3 (208 ± 11 versus 250 ± 14 g, p NS, experiment 1 (Table 4), and 231 ± 16 versus 297 ± 13 g, $p < 0.05$, experiment 3) (Table 6). When these values are corrected for the weight of the skin strips, hypox animals showed significantly greater breaking strength than the intact rats in each experiment. Also, the hypox rats accumulated as much (experiments 1 and 3) or more (experiment 2, 1739 ± 167 versus 1338 ± 94 μg, $p < 0.05$) reparative collagen in the sc implanted polyvinyl alcohol sponges than the intact rats. The treatment of hypox rats with bGH increased significantly the weight of the skin strips, wound breaking strength, and the

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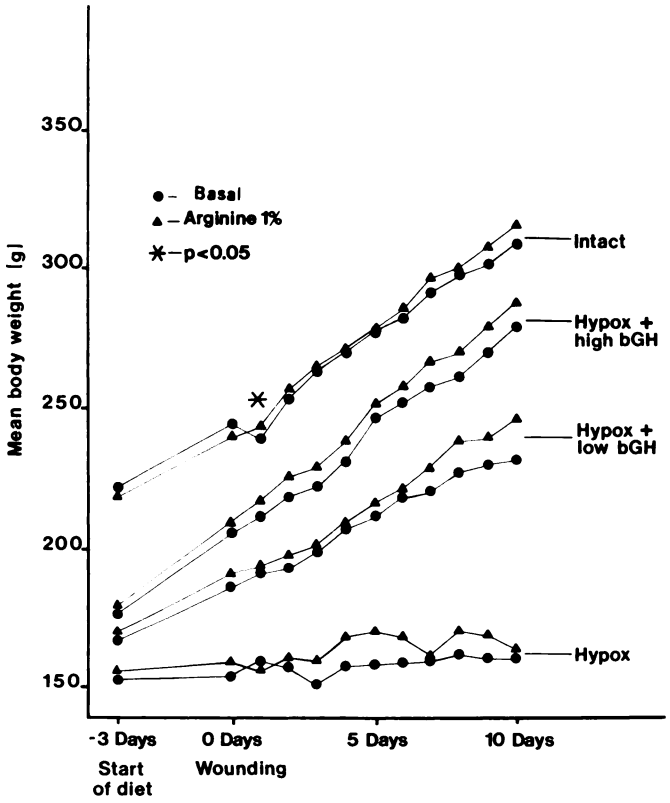


FIG 1. Body weight curves of animals in experiment 2. See text for doses and schedules of hormonal replacements.

TABLE 4
Effect of dietary arginine supplements on wound healing in experiment 1

| | | Wt of skin strips* | p | Breaking strength | p | μg hydroxyproline/ 100 mg sponge | p |
|---|---|---|---|---|---|---|---|
| A | Intact + basal | 0.98 ± 0.02 | <0.01 | 250 ± 14 | <0.05 | 2587 ± 113 | <0.005 |
| B | Intact + Arg† | 1.06 ± 0.02 | | 314 ± 20 | | 3305 ± 200 | |
| C | Hypox‡ + basal | 0.47 ± 0.01 | NS | 208 ± 11 | NS | 2278 ± 124 | NS |
| D | Hypox + Arg | 0.48 ± 0.01 | | 216 ± 10 | | 2413 ± 298 | |
| A vs C§ | | | <0.001 | | <0.05 | | NS |
| B vs D | | | <0.001 | | <0.001 | | <0.005 |

* Mean ± SEM.
† 1% arginine HCl supplements given 0.5% mixture in the diet and 0.5% solution for drinking.
‡ Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt) and testosterone propionate (750 μg).
§ Compared by analysis of variance method.

amount of reparative collagen in the subcutaneously implanted sponges; the latter two values were significantly higher than those of the intact controls fed the basal diet (experiments 2 and 3; growth hormone was not given in experiment 1).

Arginine supplementation did not affect the weight of the skin strips, wound breaking strength, or the amount of hydroxyproline in the sc implanted sponges of the hypox rats, whether or not they received growth hormone.

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TABLE 5
Effect of dietary arginine supplementation on wound healing in experiment 2

| | Wt of skin strips* | p | Breaking strength | p | μg OH-proline/100 mg sponge | p |
|---|---|---|---|---|---|---|
| | *g* | | *g* | | | |
| A Intact + basal | 0.83 ± 0.02 | <0.01 | 218 ± 16 | <0.005 | 1338 ± 94 | <0.001 |
| B Intact + Arg† | 0.96 ± 0.02 | | 297 ± 18 | | 2642 ± 160 | |
| C Hypox‡ + basal | 0.41 ± 0.01 | NS | 240 ± 13 | NS | 1739 ± 167 | NS |
| D Hypox + Arg | 0.45 ± 0.02 | | 264 ± 15 | | 1876 ± 137 | |
| E Hypox + low bGH§ + basal | 0.72 ± 0.02 | NS | 373 ± 20 | NS | 2110 ± 130 | NS |
| F Hypox + low bGH + Arg | 0.72 ± 0.02 | | 327 ± 20 | | 2470 ± 318 | |
| G Hypox + high bGH‖ + basal | 0.78 ± 0.02 | NS | 301 ± 26 | NS | 2269 ± 212 | NS |
| H Hypox + high bGH + Arg | 0.81 ± 0.02 | | 331 ± 20 | | 2308 ± 117 | |
| A vs C¶ | | <0.001 | | NS | | <0.05 |
| A vs E | | <0.01 | | <0.001 | | <0.001 |
| A vs G | | NS | | <0.001 | | <0.001 |
| B vs D | | <0.001 | | NS | | <0.005 |
| C vs E | | <0.001 | | <0.001 | | NS |
| C vs G | | <0.001 | | <0.01 | | <0.05 |

* Mean ± SEM.
† 0.5% supplementation in the diet and 0.5% solution for drinking.
‡ Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt) and testosterone propionate (750 μg).
§ bGH (0.25 IU/100 g body wt once daily).
‖ bGH (0.25 IU/100 g body wt three times daily).
¶ Compared by analysis of variance method.

TABLE 6
Effect of dietary arginine supplementation on wound healing in experiment 3

| | Wt of skin strips* | p | Breaking strength | p | μg OH-proline/100 mg sponge | p |
|---|---|---|---|---|---|---|
| | *g* | | *g* | | | |
| A Intact + basal | 0.70 ± 0.02 | <0.005 | 297 ± 13 | <0.03 | 2111 ± 167 | <0.05 |
| B Intact + Arg† | 0.82 ± 0.01 | | 355 ± 23 | | 2913 ± 287 | |
| C Intact + bGH‡ + basal | 0.61 ± 0.02 | <0.005 | 268 ± 16 | <0.001 | 1907 ± 204 | <0.02 |
| D Intact + bGH + Arg | 0.71 ± 0.02 | | 366 ± 23 | | 2684 ± 172 | |
| E Hypox§ + basal | 0.46 ± 0.02 | NS | 231 ± 15 | NS | 2138 ± 355 | NS |
| F Hypox + Arg | 0.43 ± 0.02 | | 262 ± 17 | | 1828 ± 212 | |
| G Hypox + high bGH ‖ + basal | 0.81 ± 0.02 | NS | 353 ± 21 | NS | 3535 ± 296 | NS |
| H Hypox + high bGH + Arg | 0.82 ± 0.02 | | 300 ± 23 | | 3073 ± 130 | |
| A vs E¶ | | <0.001 | | <0.05 | | NS |
| B vs D | | <0.02 | | NS | | NS |
| B vs F | | <0.001 | | <0.001 | | <0.005 |
| C vs G | | <0.001 | | <0.001 | | <0.001 |
| E vs G | | <0.001 | | <0.001 | | <0.001 |
| F vs H | | <0.001 | | <0.05 | | <0.001 |

* Mean ± SEM.
† 0.5% supplementation in the diet and 0.5% solution for drinking.
‡ bGH (0.5 IU/100 g body wt three times daily).
§ Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt), and testosterone propionate (750 μg).
‖ bGH (0.25 IU/100 g body wt three times daily).
¶ Compared by analysis of variance method.

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TABLE 7
Effect of 1% arginine supplements on the thymus wt (g) of wounded rats

| Group | Arginine supplement* | bGH | Experiment 2 | p | Experiment 3 | p |
|---|---|---|---|---|---|---|
| Intact | 0 | 0 | 459 ± 45† | <0.05 | 460 ± 25 | <0.01 |
| | + | 0 | 591 ± 25 | | 581 ± 27 | |
| Intact | 0 | 0.5 IU/100 g body wt/tid | | | 458 ± 30 | <0.02 |
| | + | | | | 612 ± 49 | |
| Hypox‡ | 0 | 0 | 58 ± 7 | NS | 43 ± 5 | NS |
| | + | 0 | 82 ± 16 | | 40 ± 5 | |
| Hypox | 0 | 0.25 IU/100 g body wt/ | 286 ± 41 | | | |
| | + | od | 331 ± 30 | | | |
| Hypox | 0 | 0.25 IU/100 g body wt/ | 364 ± 30 | NS | 372 ± 21 | NS |
| | + | tid | 435 ± 28 | | 401 ± 18 | |

* 0.5% arginine HCl in both basal diet and drinking water.
† Mean ± SEM.
‡ Daily injections of ACTH (0.5 IU), L-thyroxine (1.1 μg/100 g body wt) and testosterone propionate (750 μg).

*Thymus weight*

In both saline- and bGH-injected intact rats, supplemental arginine had a significant thymotropic effect as determined by thymus weight 10 days postwounding (**Table 7**). Administration of bGH to intact rats (fed the basal diet or arginine supplemented) did not increase thymic weight.

Hypophysectomy led to marked thymic involution which was not alleviated by administration of ACTH, L-thyroxine, and testosterone propionate. The addition of bGH to the hormonal replacement of hypox rats partially restored thymic weight in a dose-dependent manner.

No statistically significant effect of supplemental arginine HCl on thymic weight was observed in hypox rats whether or not they were growth hormone-supplemented (Table 7) although thymic weight was somewhat greater when the arginine supplement was given the latter groups.

## Discussion

These experiments show that 1% dietary arginine HCl supplementation (0.5% in the food and 0.5% in the drinking water) of wounded intact rats ingesting a commercial diet which contains 1.8% arginine and supports normal growth, reproduction, and longevity of intact rats has the following beneficial effects: *1*) lessens the immediate postinjury weight loss; *2*) accelerates wound healing as assessed by the breaking strengths of dorsal skin incisions and accumulation of reparative collagen in sc implanted polyvinyl alcohol sponges; and *3*) has a marked thymotropic effect as assessed by thymus weight. These results confirm our previous findings (4, 5). We have reported elsewhere that supplemental arginine not only lessens the loss of thymic weight after injury, but also lessens the decrease in thymic lymphocytes counts and increases the in vitro mitogenic reactivity of thymic lymphocytes from healthy and injured rodents (13). These various studies support the conclusion that supplemental dietary arginine modulates certain important posttraumatic phenomena and lessens some of the negative aspects of injury.

Our experiments with hypox rats show that an intact, responsive hypothalamic-pituitary axis is necessary for these effects of supplemental arginine to become manifest in the postinjury period: *supplemental arginine had no effect on any of the parameters studied in hypox rats supplemented with fixed doses of ACTH, L-thyroxine, testosterone propionate, and with or without growth hormone.* As anticipated, administration of bovine growth hormone to hypox rats had a strong positive effect on wound breaking strength, on reparative collagen accumulation in the sc implanted sponges, and on thymus weight. The bGH-treated hypox rats gained weight nor-

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mally, but failed to respond to arginine supplementation by accelerated gain in wound strength or by an increase in thymic weight. However, reparative collagen accumulation in arginine-supplemented intact and growth hormone-supplemented hypox rats is equivalent. With the schedule used, ie, for 3 days pre- and 3 days postwounding, bGH failed to influence wound healing or thymic weight in intact rats; however, a significant mitigating effect on weight loss was noted during the 1st day postwounding. In an experiment similar to those reported herein, we have found that administration of porcine growth hormone to intact and hypox rats exerts effects similar to those of bGH.

We had speculated that these effects of arginine may be due in part to its secretagogue activity, especially in stimulating pituitary growth hormone secretion. There is little information about how orally ingested arginine affects pituitary or pancreatic hormone release, but we believe that the difference in the route of arginine administration will not produce significantly quantitative differences; most of the ingested arginine is not degraded in the gastrointestinal tract but absorbed as such and the rate of hepatic arginine degradation allows for increases in systemic circulating levels of arginine. In this regard, it should be recalled that the route of administration, iv or oral, produces quantitative but not qualitative differences for some agents involved in stimulating the secretion of other hormones, eg, glucose, leucine, or tolbutamide for insulin. Moreover, it has recently been found that dietary arginine rapidly permeates the brains of rats.

The failure of the administration of bGH given to intact rats to duplicate the effects on wound healing of supplemental arginine given to intact rats does not rule out the possibility that the arginine effect is mediated in part by a growth hormone secretagogue action: *1*) the experimental administration of bGH three times daily to intact animals may not be biologically equivalent to the pituitary stimulation resulting from the secretagogue action of the arginine incorporated in the diet and drinking water and which the animals ingest ad libitum; *2*) the doses of growth hormone we used may not have been optimal for this purpose. Growth hormone has well known thymotropic effects (14) but the thymus glands in the intact rats given bGH in our experiments were not larger than the thymus glands of the intact rats not given bGH. This may be the result of the amount of growth hormone given. That the bovine hormone preparation we used was active is evident from its marked growth-promoting thymotropic and wound healing effects when given to the hypox rats.

When intact rats were given bGH (4 mg/day) for 7 days postlaparotomy, Prudden et al (15) found a small but statistically significant increase in wound breaking strength and strong anticatabolic activity assessed by body weight gain. Kowalewski and Young (16) administered bGH (1 mg/kg body weight) for 3 wk to intact rats that underwent 3 cm dorsal skin incisions and found significant increases in wound hydroxyproline content. We believe that the failure of administration of growth hormone to simulate the actions of supplemental arginine in our experiments may reflect timing of administration and quantitative differences, not a qualitative difference.

We have discussed elsewhere some of the other mechanisms by which supplemental arginine may exert its wound healing and thymotropic effects (4). These include its role as a percursor for proline and hydroxyproline (arginine→ornithine→glutamic semialdehyde → proline) and for energy-rich phosphoguanidines such as creatinine phosphate.

A new and unexpected finding of our experiments is that wound repair in hypox rats treated with ACTH, thyroxine, and testosterone propionate is generally as rapid as that of intact rats as judged by breaking strengths of the healing skin incisions (although their skin is much thinner) and the hydroxyproline contents of the sc implanted polyvinyl alcohol sponges. When such hypox rats are also given bGH, the breaking strengths of the skin incisions and the hydroxyproline contents of the implanted sponges are increased significantly to values greater than the corresponding values for intact rats.

We hypothesize that the pituitary of injured animals secretes (induces) both pro- and antiwound healing factors, and that our fixed dose hormonal treatment of the hypox rats with ACTH, L-thyroxine, testosterone propionate, and with and without growth hormone may "replace" principally pro-

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wound healing factors. This would be analogous to the observation of Goodson and Hunt (17) during their experiments with streptozotocin-diabetic rats; when such rats are treated with insulin promptly after wounding, the breaking strength of their skin incisions increased faster than that of nondiabetic rats treated with insulin; the basis for their observation is not known.

Our hypothesis that the pituitary of injured animals secretes both pro- and antihealing factors raises the possibility that the effect of supplemental dietary arginine on wound healing in intact rats may be due in part to an increase in the secretion of the postulated prohealing factors, to blocking of the postulated antiwound healing factors, or to both. In this respect, growth hormone behaves as a prowound healing factor, since its administration to hypox rats given ACTH, thyroxine, and testosterone results in accelerated wound healing (increased wound breaking strength and greater accumulation of reparative collagen in the sc implanted polyvinyl alcohol sponges). In fact, the rate of wound healing of hypox rats given growth hormone is faster than that of intact rats.

The failure of the hypox rats given growth hormone to respond to supplemental arginine may reflect the fact that no additional growth hormone could be secreted by them.

In conclusion, our study indicates that a dietary supplement of arginine has a number of important beneficial effects for injured rats. Furthermore, an intact and responsive hypothalamic-pituitary axis is necessary for the manifestation of the effects of supplemental arginine. We suggest that supplemental arginine may provide a safe nutritional means to improve wound healing and thymic function in injured and stressed humans.[6]

## References


1. Dale G, Young G, Latner AL, Goode A, Tweedle D, Johnston IDA. The effect of surgical operation on venous plasma free amino acids. Surgery 1977;81:295–301.
2. Furst P, Bergstron J, Kinney JM, Vinnars E. Nutrition in post-operative catabolism. In: Richards JR, Kinney, JM, eds. Nutritional aspects of care in the critically ill. Edinburgh: Churchill Livingston, 1977:389–410.
3. Freund H, Hoover HC Jr, Atamian S, Fischer JE. Infusion of branched chain amino acids in postoperative patients. Anticatabolic properties. Ann Surg 1979;198:18–23.
4. Seifter E, Rettura G, Barbul A, Levenson SM. Arginine: an essential amino acid for injured rats. Surgery 1978;84:224–30.
5. Barbul A, Rettura G, Levenson SM, Seifter E. Arginine: a thymotropic and wound healing promoting agent. Surg Forum 1977;28:101–3.
6. Sitren HS, Fisher M. Nitrogen retention in rats fed diets enriched with arginine and glycine. Br J Nutr 1977;37:195–214.
7. Franchimont P, Burger H. Human growth hormone and gonadotrophins in health and disease. New York, NY: American Elsevier Publ Co, 1975:45–77.
8. Floyd JR Jr, Fanjans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. J Clin Invest 1966;45:1487.
9. Rocha DM, Faloona GR, Unger RM. Glucagon-stimulating activity of 20 amino acids. J Clin Invest 1972;51:2346–51.
10. Poumeau-Delille G. Techniques biologiques en endocrinologie experimentelle chez le rat. Paris: Masson et Cie, 1953:31–45.
11. Levenson SM, Crowley LV, Geever EF, Rosen M, Berard CW. Some studies on wound healing: Experimental methods, effects of ascorbic acid, and effect of deuterium oxide. J Trauma 1964;4:543–66.
12. Woessner JR Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch Biochem Biophys 1961;93:440–7.
13. Barbul A, Wasserkrug HL, Seifter E, Rettura G, Levenson SM, Efron G. Immunostimulatory effects of arginine in normal and injured rats. J Surg Res 1980;29:228–35.
14. Selye H. Textbook of endocrinology. Montreal, Canada; Acta Endocrinologica, 1947:211–13.
15. Prudden JF, Nishihara G, Ocampo L. Studies on growth hormone. III. The effect on wound tensile strength of marked postoperative anabolism induced with growth hormone. Surg Gynecol Obstet 1958;107:481–2.
16. Kowalewski K, Young S. Effect of growth hormone and an anabolic hormone on hydroxyproline in healing dermal wounds in rats. Acta Endocrinol 1968;59:53–66.
17. Goodson WH III, Hunt TK. Studies of wound healing in experimental diabetes mellitus. J Surg Res 1977;22:221–7.


[6] Copy of all statistical analyses of Tables 5 and 6 are available upon request from AB.

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